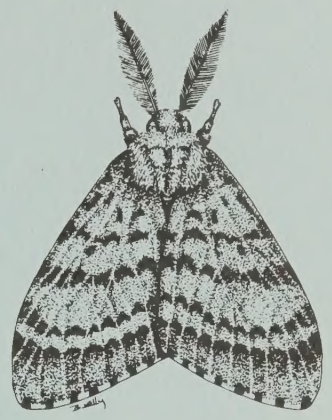

1989

United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service

Science and Technology

October 1, 1988 - September 30, 1989

Laboratory Report
Otis Methods Development Center
Animal and Plant Health Inspection Service
United States Department of Agriculture
Otis Air National Guard Base
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02542-5008

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Laboratory Report

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Project Number:	ABC 87.1.2
Project Title:	Field Determination of Predation Levels for *Coccinella septempunctata* and a Native Coccinellid in Selected Crops: Cooperative State Agreements
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leaders:	William C. Kauffman (Evaluation Project Coordinator), in conjunction with Frank Gilstrap and Jerry Michels (Texas A&M University), Timothy Kring (University of Arkansas), John Obrycki (Iowa State University), Norm Elliott and Bob Kieckhefer (ARS, Brookings, SD)

This report is a summary of the annual meeting of the Aphid Biological Control (ABC) Evaluation Team which was held in Kansas City, Missouri on October 11-13, 1989. The fundamental purpose of this Team is cooperative evaluation of predator effectiveness in aphid pest management.

APHIS APHID BIOLOGICAL CONTROL PROJECT ANNUAL EVALUATION MEETING
Kansas City, Missouri - October 11-13, 1989

Following is the summary of research reported and discussions at the 1989 Biological Control Evaluation Meeting. This is the third and final year of cooperative research agreements involving primarily *Coccinella septempunctata* (C7) which have been coordinated by Otis Methods Development Center. A comprehensive report summarizing these three years of research aimed at evaluating the impact of C7 of aphid populations will be compiled in the near future. Throughout the past three years this group of scientists from APHIS, ARS, and university and state agencies has contributed valuable agricultural and ecological data on the impact of C7 on various aphid species. In addition, our efforts have significantly expanded the previous knowledge of ecological interactions of this introduced predator with non-target organisms, particularly its compatibility with indigenous natural enemies. The final report will further clarify the role of C7 in aphid suppression in agroecosystems and, in addition, provide the foundation for evaluating the impact of parasitoids and predators deployed in classical biological control of the Russian wheat aphid (RWA), *Diuraphis noxia*. To this end, a group of scientists knowledgeable with RWA will be assembled in the next few months to organize and initiate the evaluation of APHIS' Aphid Biological Control (ABC) Project targeted for this new pest of small grains.

COOPERATIVE RESEARCH BETWEEN USDA-APHIS AND UNIVERSITIES

Field Cage Study:

These field investigations of C7 and the native ladybeetle, *Hippodamia convergens* (Hc), were conducted in Arkansas, Texas and Iowa according to procedures outlined in the 1987 report and modified in 1988. At the time of the meeting, reports of research from Arkansas and Texas were interim, pending data from field studies still in progress. The objectives of these field cage (6' X 6' X 6') studies were:

1) to set standard predation levels for C7 in cages in selected crops for each state; and
2) to compare the predation efficiencies of C7 and Hc.

The experimental design was a 3 X 3 factorial which incorporated three levels of each species. First instar larvae (Arkansas, Iowa) or eggs (Texas) of each species reared at the Niles Biological Laboratory were introduced into field cages in quantities as indicated; the ratio of C7 and Hc represents the number of individuals of each predatory species on a per plant (Arkansas) or per cage (Iowa, Texas) basis. Cages which received no coccinellids were the controls. Each cage was terminated at coccinellid pupation (Arkansas and Iowa) or continued into the adult generation (Texas) unless plants had died at an earlier date. Experimental treatments were replicated 2-4 times throughout the growing season.

Tim Kring (University of Arkansas)

Field Cage Study:

Initial densities of greenbug, *Schizaphis graminum*, were ca. 50/plant with ten grain sorghum plants per cage. Again, as in 1987 and 1988, aphid densities in cages during the first replicate, initiated on 14 July, followed no logical pattern as a result of C7 and Hc added. Ants are more abundant in early replicates and they interfere with aphid predation by removing small coccinellid larvae from plants. Diazanon® was applied in these first replicates for control of ants. A possible explanation for these spurious results is that controlling ants may interfere with normal predation levels. This explanation was further supported by the significant correlation between greenbug densities and amount of insecticide in the cage.

The second replicate, initiated on 18 August, had the highest greenbug density in the control cage (Fig. 1). Despite apparent visual differences among cages, there were no significant differences among treatments. This lack of significance was apparently due to high variability in aphid densities and the small number of replications. Nonetheless, the lowest densities occurred in the cages with both predators present. It appears that Hc has a greater impact that C7 on greenbug densities in sorghum. C7 does not appear to interfere with the impact of Hc since their combined predation level is nearly additive rather than antagonistic. Ten to thirty percent of larvae survived to adulthood.

Predator-Induced Mortality and Foraging Behavior of C7:

Four to five times as many aphids were dislodged from plants by C7 foraging than were consumed by this predator. More greenbugs were dislodged from plants as aphid densities increased, whereas plant growth stage had no effect. Soil temperature was the major factor determining the probability of a dislodged aphid returning to a feeding position on a sorghum plant. The soil temperature threshold above which the incidence of aphid mortality begins to increase is 37°C. Dislodged aphids that will return to the plant re-established within three hours. Therefore, a meaningful estimate of total predator-induced mortality must add from 59% (at threshold or below) to 310% (above 37°C) to the level of predation. Although foraging by C7 and Hc increased from 7 AM to 11 PM, neither species exhibited a significant decline in foraging activity from mid-day to 7 PM (Fig. 2, preliminary data).

Jerry Michels, Frank Gilstrap and Jeff Edwards (Texas A&M University)

Field Cage Study:

Four replications of the field experiment were conducted in 1989. Replicate III (cage 4 only) and replicate IV were continuing at the time of this meeting, therefore conclusions on these replicates are incomplete.

Clearly, early suppression of greenbug is the key to aphid control under these cage conditions. Number of coccinellid larvae generally peaked between days 3 and 5, and pupation occurred between days 13 and 17. Aphid densities increased rapidly throughout the pupation interval, which represents a dramatic decline in total active predatory units. The more effective the coccinellids are at reducing aphid density prior to pupation, the more likely aphid suppression will be maintained after the adults emerge (replicates III and IV). However, this is only true if the predators do not overgraze their available resources and starve (replicate I). In the first replicate, mean greenbug density on day 13 in predator cages was 17.6% of the greenbug density in the control cage, thus representing an 88.4% reduction of aphids (Fig. 3a). Pupation of natural populations of ladybeetles occurs less synchronously over a wider interval due to a prolonged oviposition period. Open-field observations in Texas indicate that if coccinellids provide early suppression of aphids, other natural enemies will invade and regulate explosive aphid growth during coccinellid pupation. In replicate II, no coccinellid treatment significantly affected greenbug density. This lack of greenbug control was likely due to abnormally high mortality of Hc and C7 during the five days immediately following their introduction (91% and 38% respectively).

Some suppression of greenbug occurred in all cages in replicate III (Fig. 3b) except cage 6 (100 C7 : 200 Hc per cage). Doubling the number of individuals of a single species did not significantly affect the level

of prey reduction. Aphid suppression prior to pupation was more pronounced when C7 and Hc were both released than when a single species was released at either the high or low level.

Cages from replicate IV, which are still in progress, indicate that aphid suppression in all cages was significant compared to the control; however, at these higher temperatures aphids were reproducing at rates exceeding the ability of ladybeetles to consume them.

Throughout these 1989 field studies, C7 appears to equal Hc in its aphid reductive potential, and antagonism of predation levels of these native and introduced predators is not evident.

John Obrycki (Iowa State University)

Field Cage Study:

Three replicates of this 3 X 3 factorial (=27 total cages) were conducted in second year alfalfa, cv. Vernal. Pea aphid, *Acyrthosiphon pisum*, and coccinellids were sampled using a pan sampling technique. First instar coccinellids were introduced in two densities (80 and 160 per cage) on June 7. Adults began emerging on June 27 and data were recorded until July 12. Aphid densities in the control cages increased logarithmically to an average of 3000 pea aphids per cage. Pea aphid mean densities in the eight treatment cages where coccinellids were released were lower than mean densities in control cages from 12-23 June (Fig. 4). Two-way ANOVA on the aphid numbers for each sample date failed to indicate significant differences among coccinellid release treatments due to the high variability in aphid numbers among the three replicates. That is, there was no significant difference between 80 and 160 C7 and between 80 and 160 Hc (Fig. 5); between 80 C7:160 Hc and 160 C7:80 Hc; and among 160 Hc, 160 C7, 80:80 and 160:160 (Fig 6). Survival of coccinellids to adults (mean survival per cage) was lowest (1-7%) when 160 of each species were released, and highest (29-40%) when 80 individuals of each species were released. Mortality was greatest among first instars.

In conclusion, based on two years of field results of introducing ladybeetle larvae there is no statistical reduction of pea aphid densities in alfalfa due to C7 predation compared to control cages. Very high variability of aphid populations prevents statistically significant results with small numbers of replications. These two predators have similar capabilities for aphid reduction under these cage conditions. In cages receiving both predators, no clear pattern of negative interaction was observed between C7 and Hc; therefore no obvious incompatibility between these species exists.

Parasitization of Native and Exotic Coccinellids by *Dinocampus coccinellae*:

Abstract from J. Kansas Entomol. Soc., J. J. Obrycki. 1989. 62(2): 211-218.

Adults of three Nearctic coccinellid species, *Coleomegilla maculata, Cycloneda munda* and *Hippodamia convergens,* and one Palearctic species, *Coccinella septempunctata,* were suitable hosts for the braconid parasitoid *Dinocampus coccinellae*. Mean parasitoid developmental times at 22°C ranged from 30 to 33.3 days, and the percent successful parasitization varied between 30 and 57%. Only 1.5% of *D. coccinellae* emerged from *Propylea quatuordecimpunctata*, a second Palearctic species found in North America since 1968; developmental times were significantly longer (mean = 37 days) compared with those for suitable hosts. No *D. coccinellae* successfully completed development in *Hippodamia variegata*, an exotic species discovered in Canada. These comparative studies provide a basis for examining the potential *D. coccinellae*-mediated interactions among invading and indigenous coccinellid species.

Prey Suitability of Aphids for Three Introduced Coccinellids:

Abstract of paper submitted to J. Econ. Entomol., J.J. Obrycki & C.J. Orr.

Total larval development of C7 reared at 23°C on pea aphids averaged 13.1 days, which was significantly faster than development (16.0 days) on corn leaf aphids. Adult C7 from larvae reared on pea aphids were larger and weighed more than those reared on corn leaf aphids. Developmental times of Hv and P14 were not influenced by larval prey; however, adult P14 were heavier and larger when reared on pea aphids. First

instars of each coccinellid species did not feed on European corn borer eggs. Therefore, pea aphid is a highly suitable larval prey for these three predators and redistribution releases in pea aphid-infested alfalfa are appropriate. In corn agroecosystems these coccinellids can develop on corn leaf aphids, but first instars cannot utilize European corn borer eggs as an alternate food source.

Intrinsic Rate of Increase of Biotypes of P14:

David Orr and John Obrycki have submitted a computer program (Fla. Entomol.) which calculates interval estimates with which to statistically compare r_m. This analysis demonstrated differences in r_m values for Canadian, French and Turkish biotypes of P14 feeding on pea aphid (preliminary).

Epilogue

As previously noted, high variability in aphid survival and growth rates and few replications prevented statistically significant results with these aphidophagous coccinellids in large field cages. Jerry Michels, Tim Kring and John Obrycki will investigate additional ways to analyze this field data, including the possibility of collectively analyzing data from the three locations, in order to get these results into a final report and, hopefully, a refereed publication.

COOPERATIVE RESEARCH BETWEEN USDA-ARS AND USDA-APHIS

Norm Elliott and Bob Kieckhefer (ARS, Brookings, SD)

This ongoing ARS multi-crop coccinellid research project was supported in part by APHIS in the form of financial support from Otis Methods Development Center for a summer technician for the past two summers as well as a reliable supply of ladybeetles from Niles Biocontrol Laboratory.

Field Cage Studies with Exotic Coccinellid Feeding on Greenbug:

Twice during the summer of 1989, twelve 10' X 10' field cages were placed over spring wheat seedlings, then plants were infested with greenbugs. Although three coccinellid treatments (25 Hc, 62 P14, and 164 *Scymnus frontalis* [Sf]) did not significantly lower greenbug densities, in both trials greenbug populations were somewhat lower for Hc and Sf than for controls.

Laboratory Studies of *Scymnus frontalis:*

Studies were conducted to determine the effect of temperature on Sf immature developmental rates and survival, and the effect of three species of aphids (RWA, greenbug and pea aphid) as prey on reproduction and survival of Sf adults. The developmental threshold of 11.5°C for Sf larvae on RWA was somewhat higher than that of many common North American coccinellids. Sf adult survival and fecundity was unaffected by prey consumed.

Aphidophagous Insect Communities in Agroecosystems and the Impact of C7 on Native Species:

Single fields of alfalfa, small grains and corn were sampled at three locations in eastern SD. Preliminary conclusions are that C7 populations increased over 1988 levels in all three crops. C7 is now generally second in abundance to Hc (C7 went from 1% of the coccinellid assemblage in 1988 to 15% in 1989) although its abundance varies among crops. Two native coccinellids, *H. tredecimpunctata* and *C. transversoguttata,* have declined markedly over the past few years. However, it would be premature to conclude from only two years' data that this decline in indigenous predator abundance reflects displacement by C7.

Efficient Sampling Methods for Coccinellids in Small Grains:

The objective of this investigation was to determine if commonly used methods of relative sampling of coccinellids in wheat fields could be confidently related to the actual number present. Successive removal sampling was used to obtain absolute estimates of coccinellid population density in spring wheat fields. It was concluded that sweepnet and visual counting provided reliable methods for sampling adult coccinellids in wheat. Estimates obtained by sweepnet sampling can be related to absolute population density by a regression model which incorporates temperature and plant growth stage. Sweepnet sampling also provides reliable estimates of relative abundances of each coccinellid species.

Abstract of paper submitted to Can. Entomol., N. C. Elliott, R. W. Kieckhefer and W. C. Kauffman

Title: Sampling aphidophagous lady beetles in wheat fields by removal sampling, sweepnet and walking counts.

Three methods (removal sampling, sweepnet sampling, and counts taken while walking at constant velocity through a field) were used to sample populations of five lady beetle species in plots established in wheat fields. Estimates of absolute population density obtained from two 20 minute removal samples taken from each of six 5x5 m sub-plots per plot proved reliable and were used to convert estimates obtained from sweepnet sampling (180 sweeps per plot) and walking counts (36 min. per plot walking at 10 m/min.) into absolute estimates of density. For most species, population density estimates obtained by removal sampling were quite precise. However, for species with low capture efficiency and low population densities, estimates were less precise. Crop growth stage influenced the numbers of beetles caught in sweepnet catch to absolute population estimates. Values of R^2 of regressions ranged from 0.51 to 0.90, depending on species. Walking counts were influenced by temperature and aphid density and these variables were incorporated into regression models. Values of R^2 for regression relating walking counts to population density ranged from 0.63 to 0.94, depending on species.

Abstract of paper submitted to Can. Entomol., N. C. Elliott, R. Kieckhefer and W. C. Kauffman

Title: Development and evaluation of a fixed-precision level sequential sampling method for lady beetles in wheat.

A precise relationship between the mean and variance of the numbers of lady beetles seen in 2 minute visual counts made while walking at a velocity of 10 m per minute was obtained using Taylor's power law. The relationship was used to develop a sequential sampling procedure for estimation of the mean number of lady beetles per 2-min. count with known average precision (standard error/mean). Using an equation relating the number of lady beetles per m^2 to the number of lady beetles per 2-min. count and temperature, mean numbers of beetles per 2-min. count obtained by sequential sampling were converted to estimates of absolute population. The sampling plan was modified to compensate for error in estimates of power law parameters and for technical limitations on the number of samples taken. Using the modified plan, a maximum of 18 two-min. counts are conducted. Average precision varies depending on the mean number of beetles per 2-min. count. For populations of less than 1.5 beetles per 2 min., estimation is imprecise because sample sizes larger than 18 are required to achieve moderate precision. However, assuming that the sampling plan is unaffected by fluctuations in biotic and abiotic variables, with approximately 95% assurance, average precision is less than or equal to 0.25 whenever the mean number of beetles per 2 min. is greater than 1.5.

Detailed Mechanistic Model of the Population Dynamics of Hc and the English Grain Aphid:

Norm is developing a predator-prey model which incorporates various environmental, habitat and biological variables that have the greatest influence on predation levels, and thus the population interaction.

Aggregative Response by Coccinellids and the Mortality Rates of Cereal Aphids in Small Grains:

For the last two years, cages were removed from patches of small grain fields artificially infested with cereal aphids. Thereafter, coccinellid densities and aphid densities were recorded in an attempt to determine the effects of environmental factors (viz. temperature, relative humidity and rainfall) on fecundity and survival of aphids and aggregation and predation rates of coccinellids. Results are inconclusive at this point.

Reanalysis of 15 Year Historical Database of Coccinellid Communities:

Correspondence analysis of 15 years of data collected at three sites in South Dakota suggests that, despite large year-to-year and site-to-site variation, there is a systematic change in predator communities as agricultural crops change.

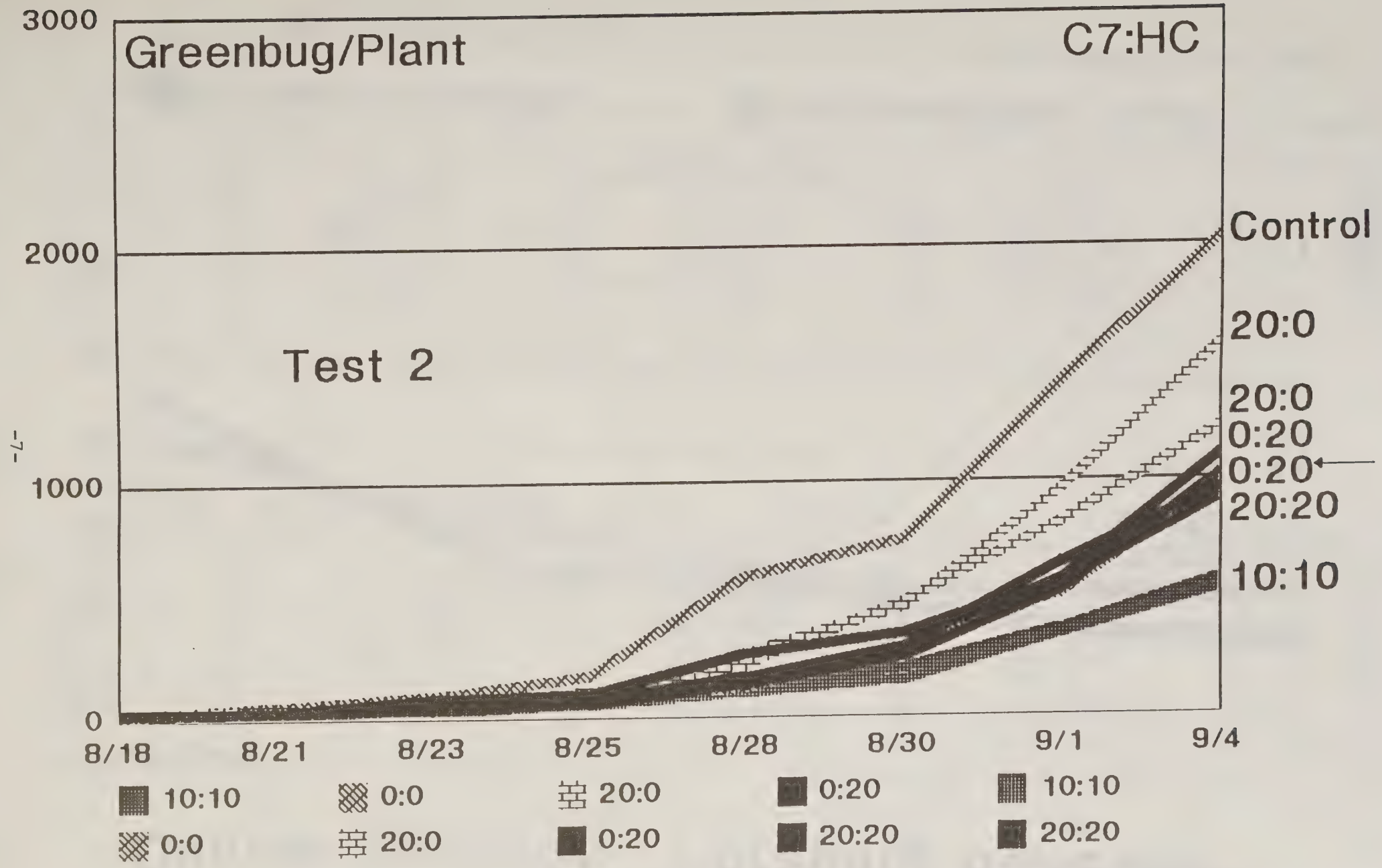


Figure 1. Greenbug densities on sorghum at various combinations of C7 and Hc, August 18 - September 4; Trial 2, Arkansas. Note: Multiply by 10 for greenbug density per cage.

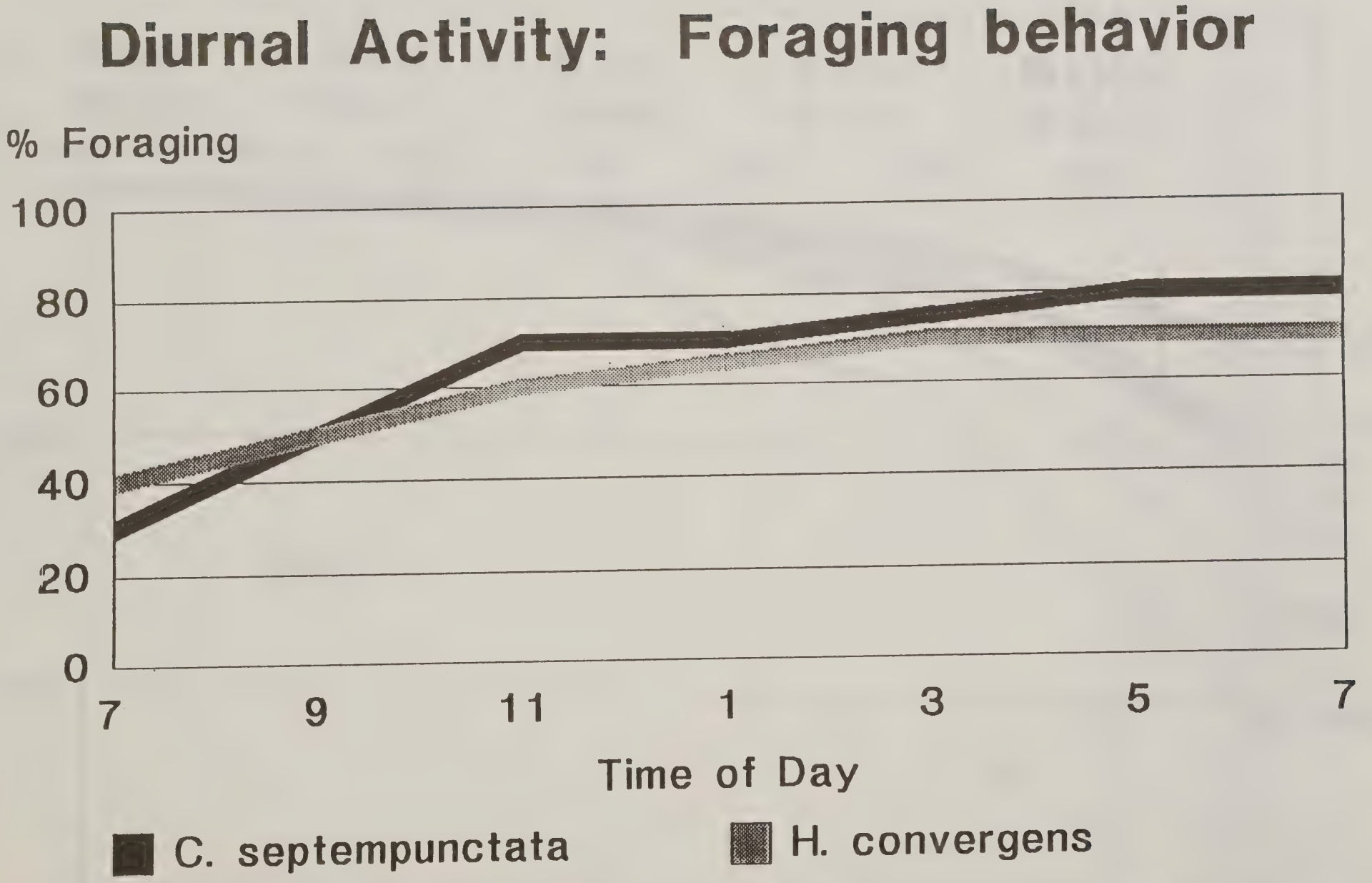


Figure 2. Diel pattern of foraging behavior of two coccinellids on young sorghum plants (5-8 leaf stage) at field conditions; Arkansas.

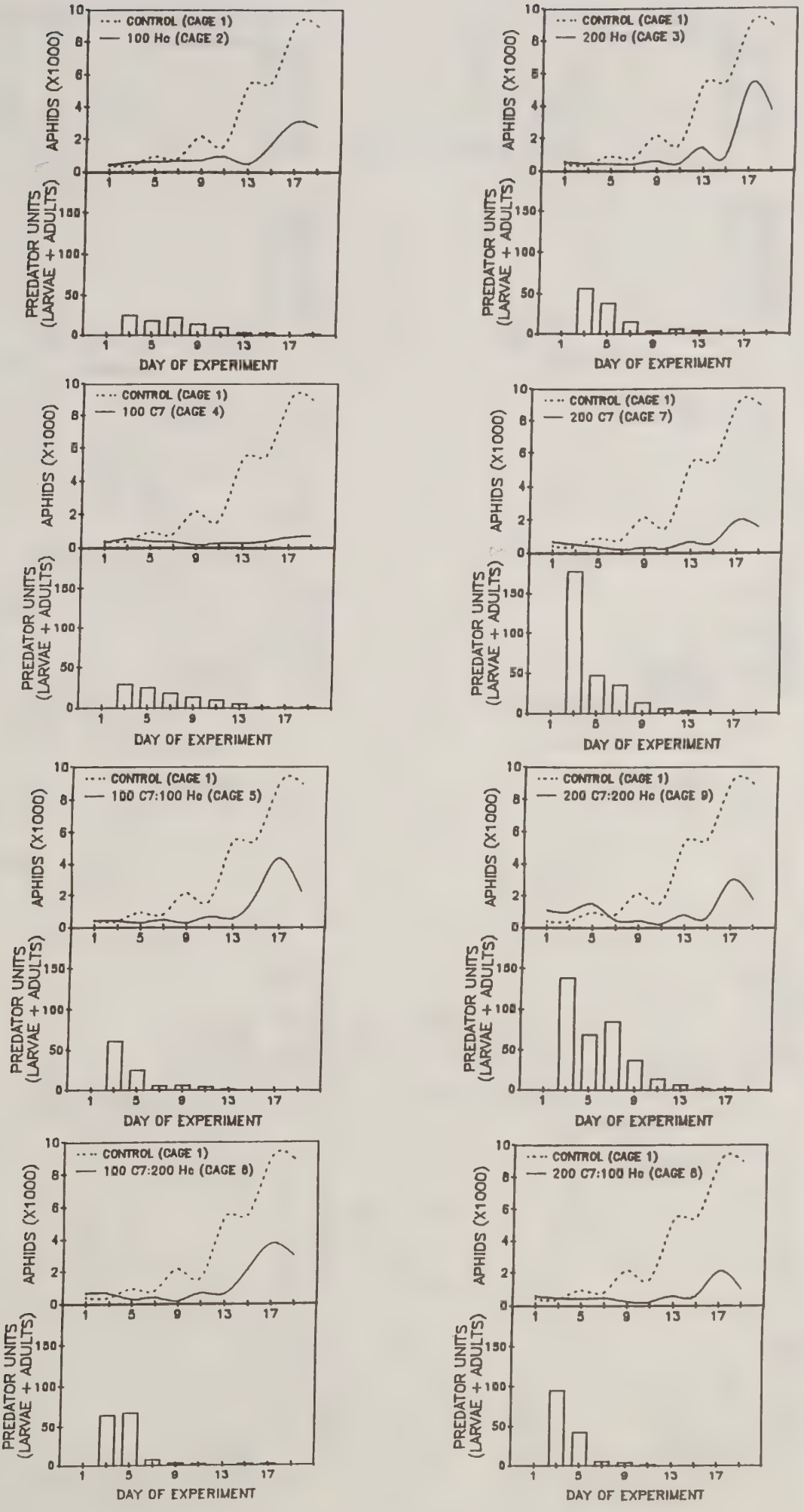


Figure 3a. Greenbug densities on sorghum at various combinations of C7 and Hc, initiated June 15; replicate I, Texas.

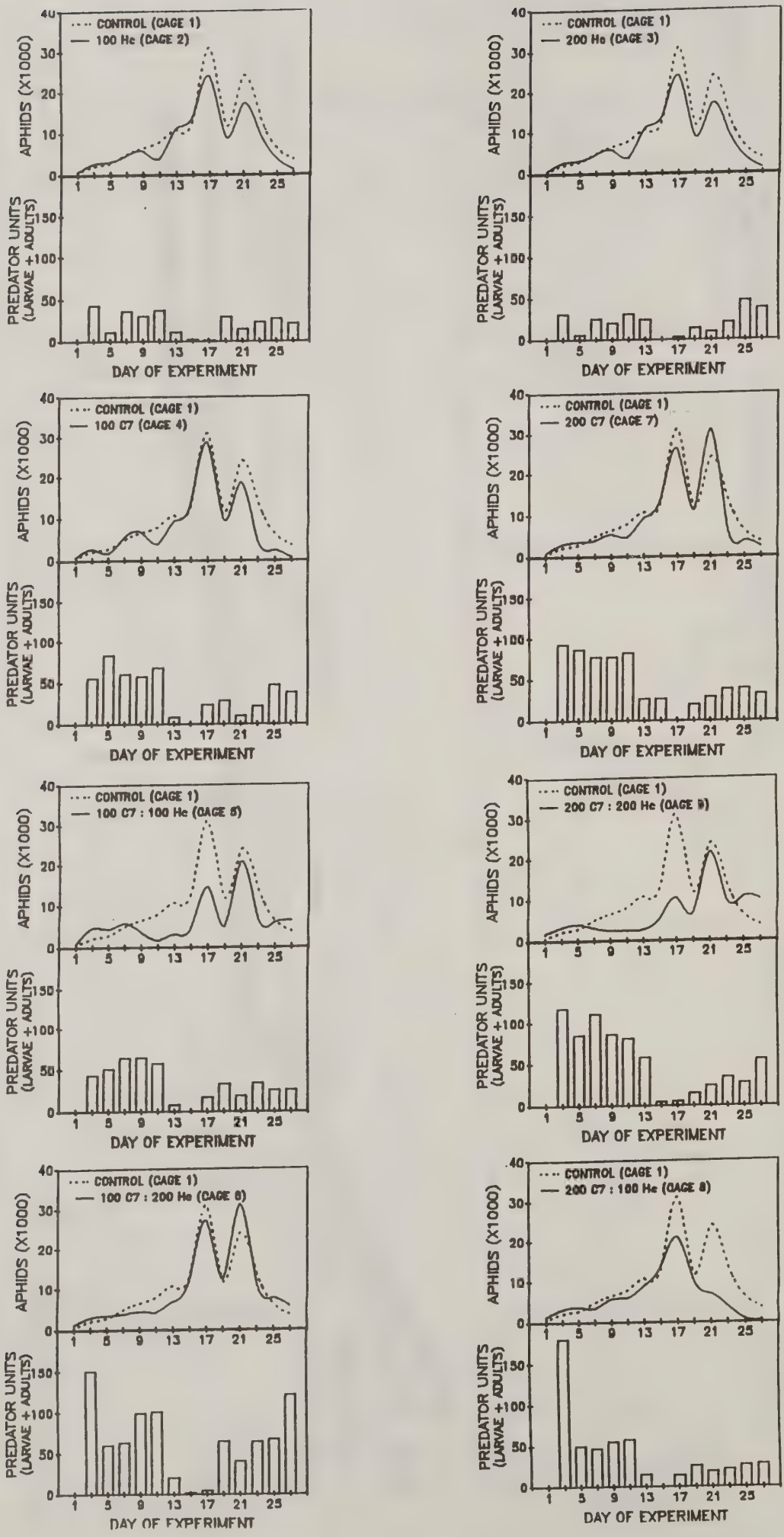


Figure 3b. Greenbug densities on sorghum at various combinations of C7 and Hc, initiated July 23; replicate III, Texas.

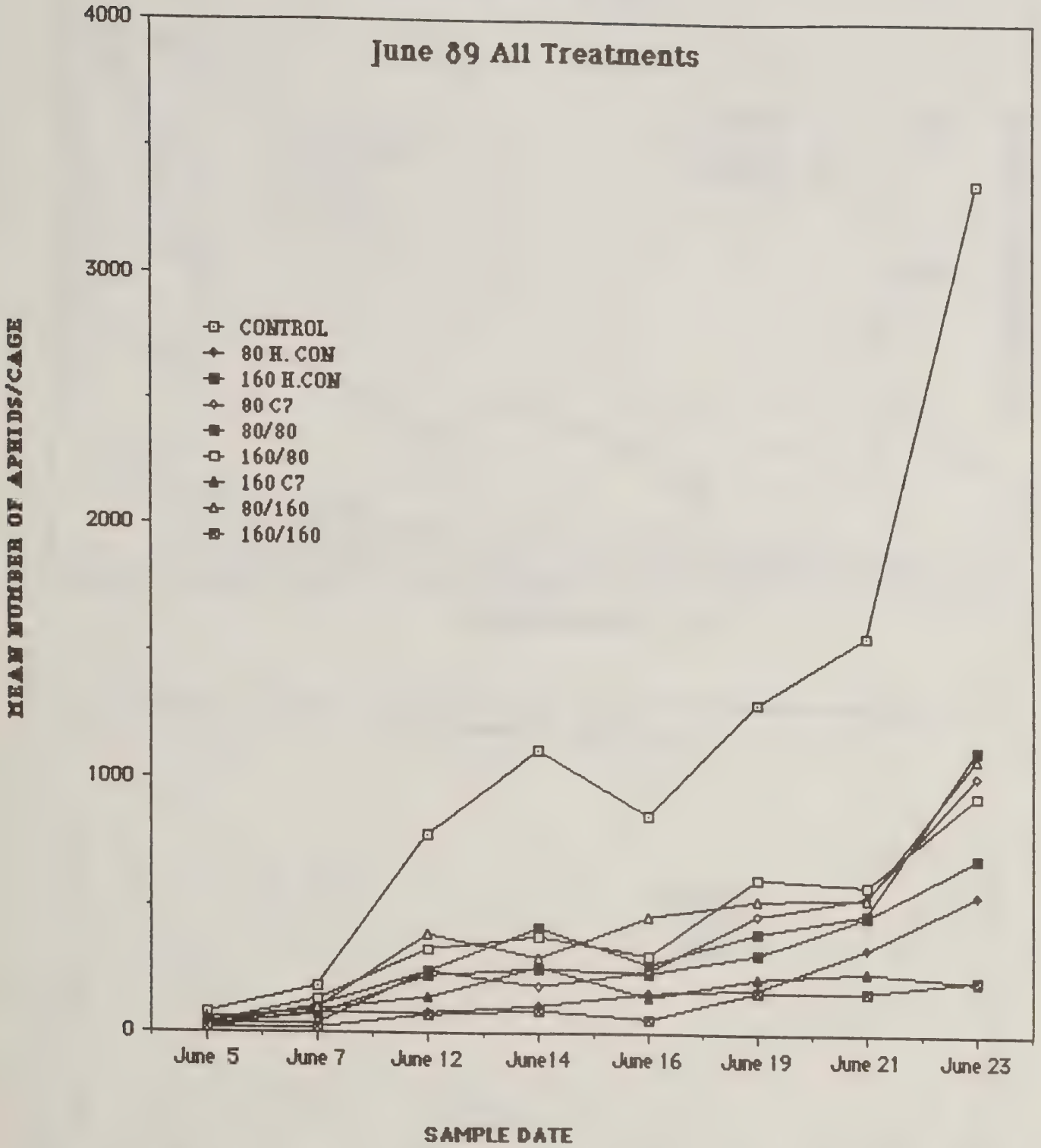


Figure 4. Pea aphid densities at various combinations of C7 and Hc compared to a control, Iowa.

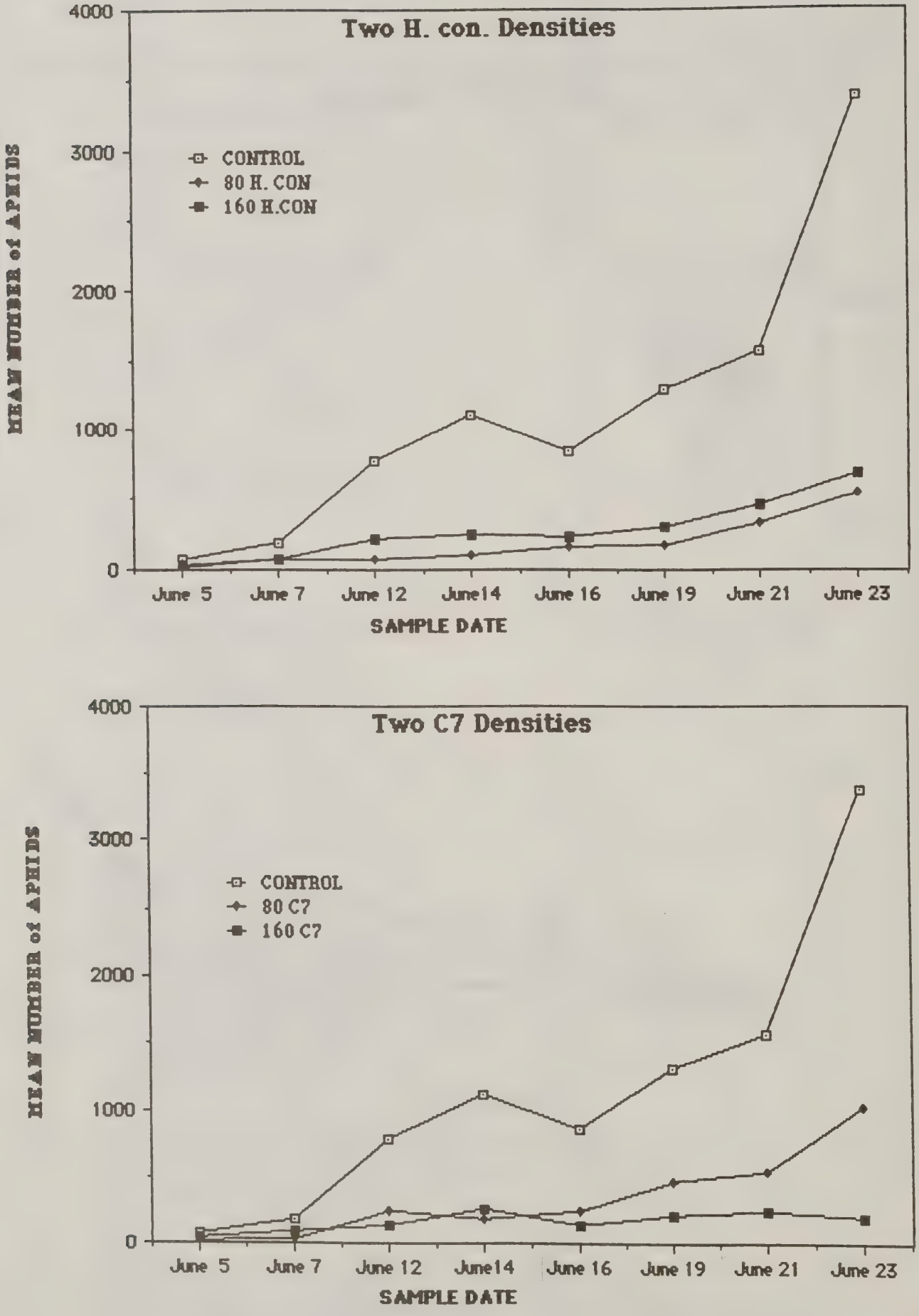
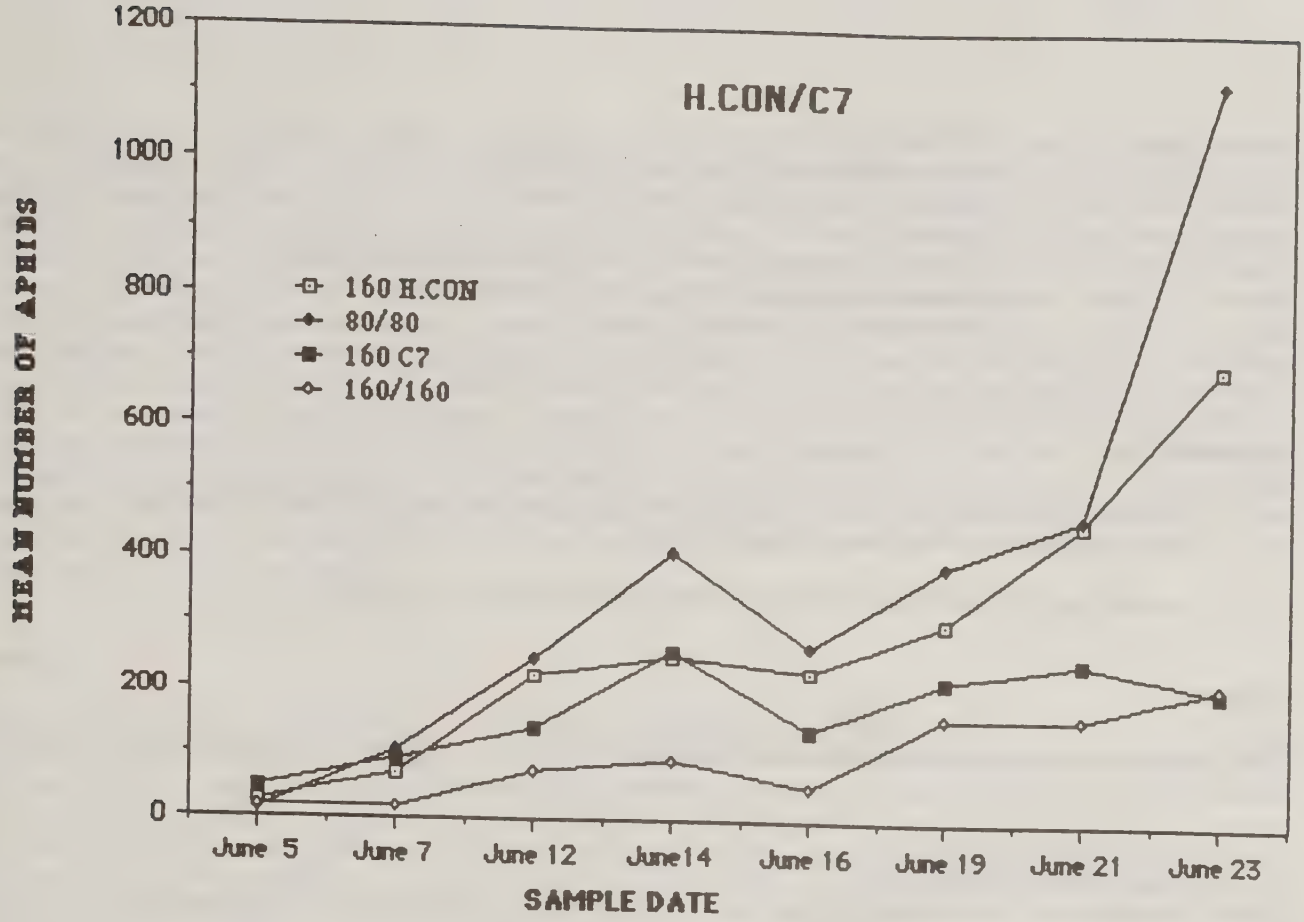


Figure 5. Pea aphid densities with single species coccinellids; Iowa.



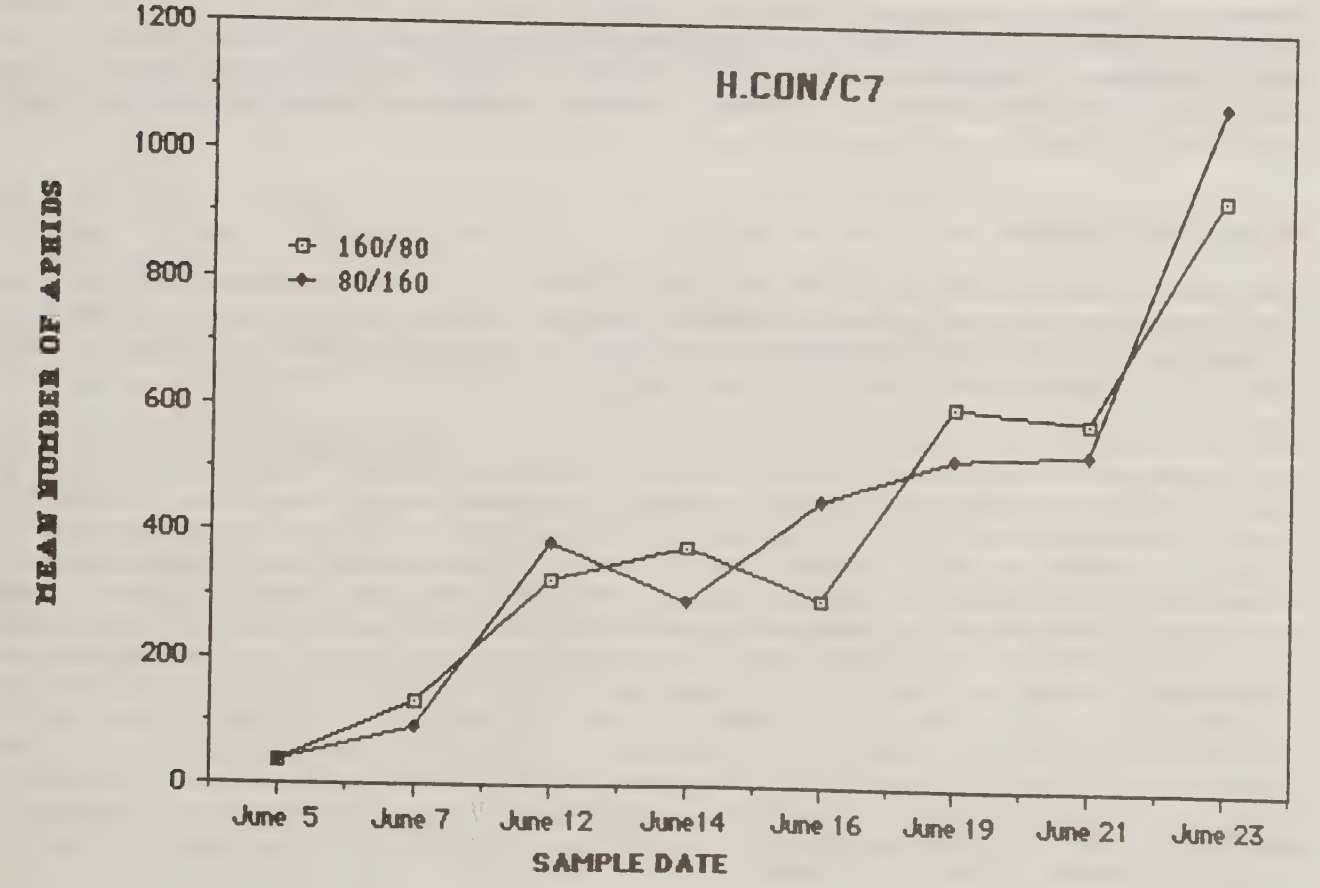


Figure 6. Pea aphid densities with combined species of coccinellids; Iowa.

Project Number: ABC 88.1.3
Project Title: C7 Predation of *Aphis fabae* in Fava Bean
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leader: William C. Kauffman, assisted by Linda Correia, Debbie Moreau, Kim Murchison, Lucia Rossoni and Albert Wurzberger

Objectives

1. Determine the ability of the lady beetle predator, *Coccinella septempunctata*, to reduce aphid densities in fava bean, *Vicia fabae*, and

2. Assess how this aphid suppression affects plant growth and crop yield.

Rationale

Three experimental or comparison methods are appropriate for a quantitative evaluation of natural enemies, including:

1. the addition method,
2. the exclusion method, and
3. the interference method (DeBach, Huffaker and MacPhee 1976)

It was our desire to conduct this study with natural populations of both prey and predators with minimal disturbance. Therefore, we designed this field experiment using daily hand removal of C7 (i.e., interference method) to contrast aphid densities and fava bean growth between plots with C7 remaining intact and plots with C7 removed. This crop was chosen for its reliability in harboring natural infestations of aphids, economic value as a fresh-market crop, and host plots and agro-habitats conducive for C7 aggregation and predation of aphids.

Materials and Methods

Field research was conducted at New Alchemy Institute, which is a research/educational facility on Cape Cod, Massachusetts that promotes sustainable agriculture and organic gardening. The New Alchemy site represents a small farm agroecosystem which maximizes the role of beneficial insects within the context of low inputs of synthetic chemicals amid a rich ecological diversity of flora and insect fauna.

Six plots with paired treatments (i.e., control and C7 removal) were spatially separated by a minimum of 200 feet. Planting and crop maintenance procedures were standardized in all plots. Plots were 20 feet x 20 feet (ca. 6m x 6m) with rows oriented east to west, spaced 24 inches apart and ca. 40 seeds/row. A 5-foot high barrier of polyethylene and nylon (Vispore®, Gardener's Supply, Burlington, Vermont) extended east to west through the middle of the plot to subdivide the plot. This Vispore barrier allowed for air movement between subplots and repelled water, and was thus superior to the cloth barrier used in 1988. In addition, in this second year of the experiment a ground-level barrier of 12-inch high metal flashing (8 inches above ground) circumvented each C7 removal subplot; the top 3-4 inches of flashing was sprayed with a dry-film lubricant (Fluoroglide®, Performance Plastics, Wayne, New Jersey) to prevent entry of larval stages of C7 into this removal subplot. Each of the paired subplots was randomly assigned as a control (C7 included) or experimental (C7 removed) treatment. Data collection was identical to the procedures used in 1988. Following germination, plots were surveyed semi-weekly for the presence of aphids and C7 in order to determine the suitable time to initiate intensive data collection. We initiated alternate day censusing of aphids and C7, and C7 were removed daily when plants were small (5 inches tall). Data were recorded daily between 8:00 AM and 12 noon until harvest.

In each subplot, the number of aphids were recorded from all plants in a randomly selected meter of each row. All stages of C7 were recorded from both sides of the Vispore barrier and were removed contemporaneously from the experimental subplot. These daily activities presumably provided similar levels of habitat disturbance in both control and removal subplots. The middle barrier and the metal flashing barrier around the removal side were designed to minimize cross-movement of C7 between adjacent subplots. Plant vigor was assessed throughout the experiment by measuring various plant parameters including plant height and the number of nodes, flowers and pods. At early harvest pods, stems and leaves were weighed, both as fresh and dry tissues. For dry weight determinations, excised plant parts were desiccated in a drying oven at 70°C for 18 hours. Pods were categorized as large (>5 inches in length), medium (2-5 inches) or small (<2 inches). Aphids densely located on pods produce large quantities of honeydew, which is a favorable growing medium for sooty mold, anthracnose and other plant pathogens. Casual observation suggesting a possible link between the occurrence of these secondary pathogens and high densities of *A. fabae* was confirmed by 1988 data when aphid levels were more than double the levels recorded in 1989. Therefore, quality of pods was again assessed in 1989 on the basis of blemishes associated with these pathogens.

Results

Data were subjected to analysis of variance (AOV), paired t-test, $P<0.05$. Removal of C7 significantly increased aphid densities during a 6-day interval in mid-season when large larvae were present (Fig. 1). However, aphid levels in mid-season in control plots began declining after June 28 culminating to a low level on July 4 not significantly different from prey numbers in the C7 removal subplots. This rapid collapse of the aphid was apparently due to aphid emigration as well as predation by a syrphid fly (species yet undetermined) and parasitism. Daily removal of C7 effectively reduced numbers of this predator (daily mean of 5.8 individuals per control subplot versus 3.0 per removal subplot) while the abundances of other coccinellids did not differ (daily mean of 0.7 vs. 0.6).

Fava bean plots from which C7 were removed, resulting in higher aphid densities, produced smaller plants as indicated by lower biomass of pods, stems and leaves than the control plants (Fig. 2). Plants from which the predator was removed did not mitigate aphid-inflicted water and nutrient losses by increasing vegetative growth in stems and leaves as had been suggested by preliminary data in 1988 when aphid levels were higher. There was no effect of treatment on pod blemishes by plant pathogens in 1989.

At the end of season harvest, plants from plots where C7 had been removed produced fewer pods (Fig. 3) and lower fresh and dry weights of seeds (Fig. 4). Daily removal of C7 from fava bean plants resulted ultimately in a 16.7% decrease in crop yield compared to control plots.

In conclusion, natural populations of C7 at this field location provided suppression of bean aphid in fava bean at a critical period of plant development. This level of predation was sufficient to demonstrate an economic benefit of C7 in the control of this aphid in fava bean. High levels of aphid predation, particularly by large larvae, decreased plant damage from aphid herbivory, thus resulting in larger plants and greater numbers of pods and seeds.

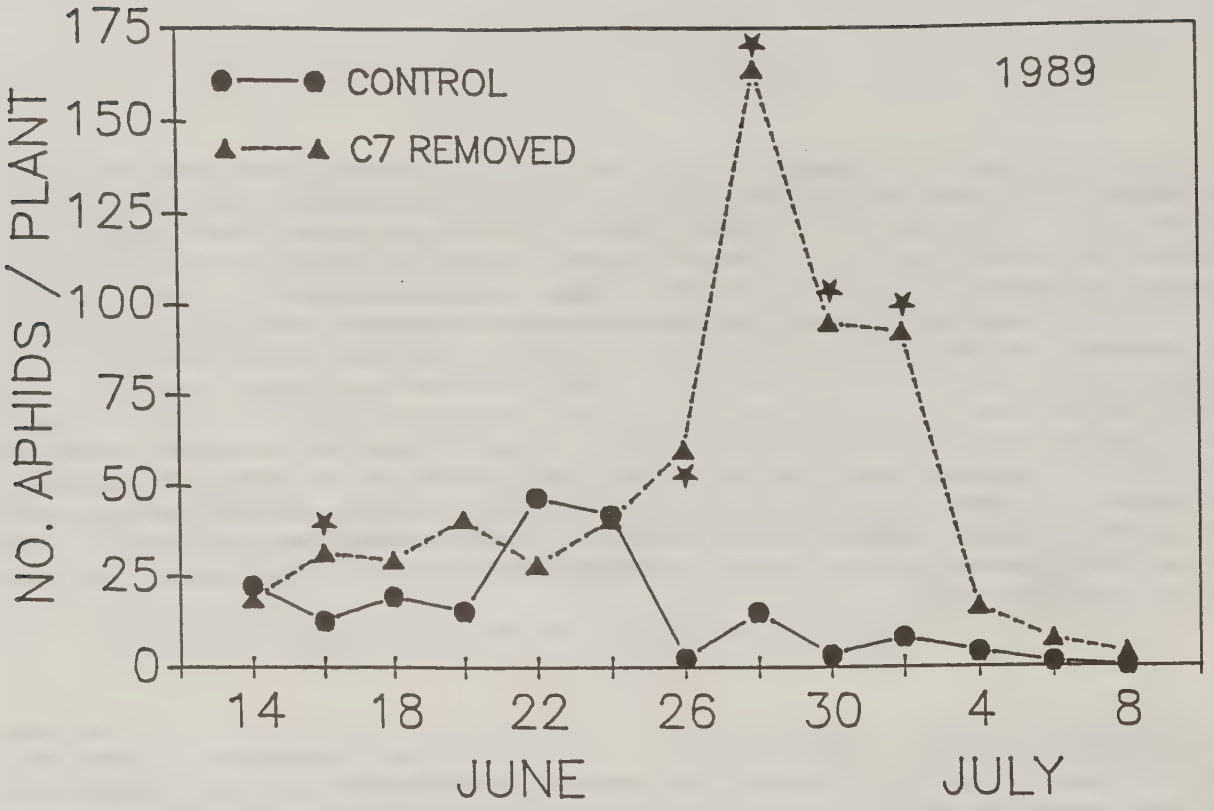


Fig. 1. Mean density of *Aphis fabae* in field plots in which *C. septempunctata* was removed versus control plots; an * indicates significant differences in the means, ANOVA, paired t tests, $P<0.05$.

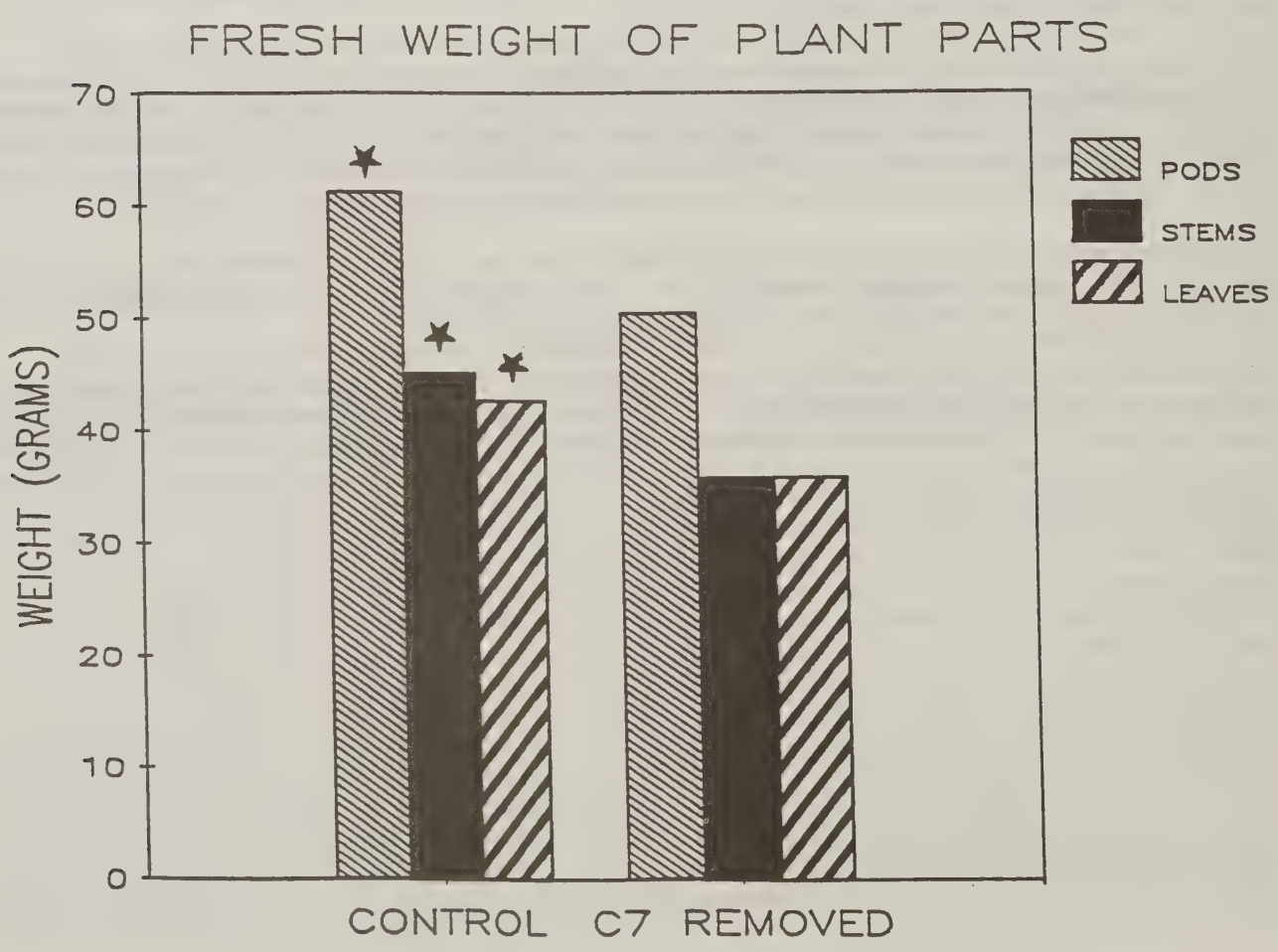


Fig. 2. Mean fresh weights of pods, stems and leaves of fava beans with *C. septempunctata* removed or intact; * indicates significance ($P<0.05$) between treatments.

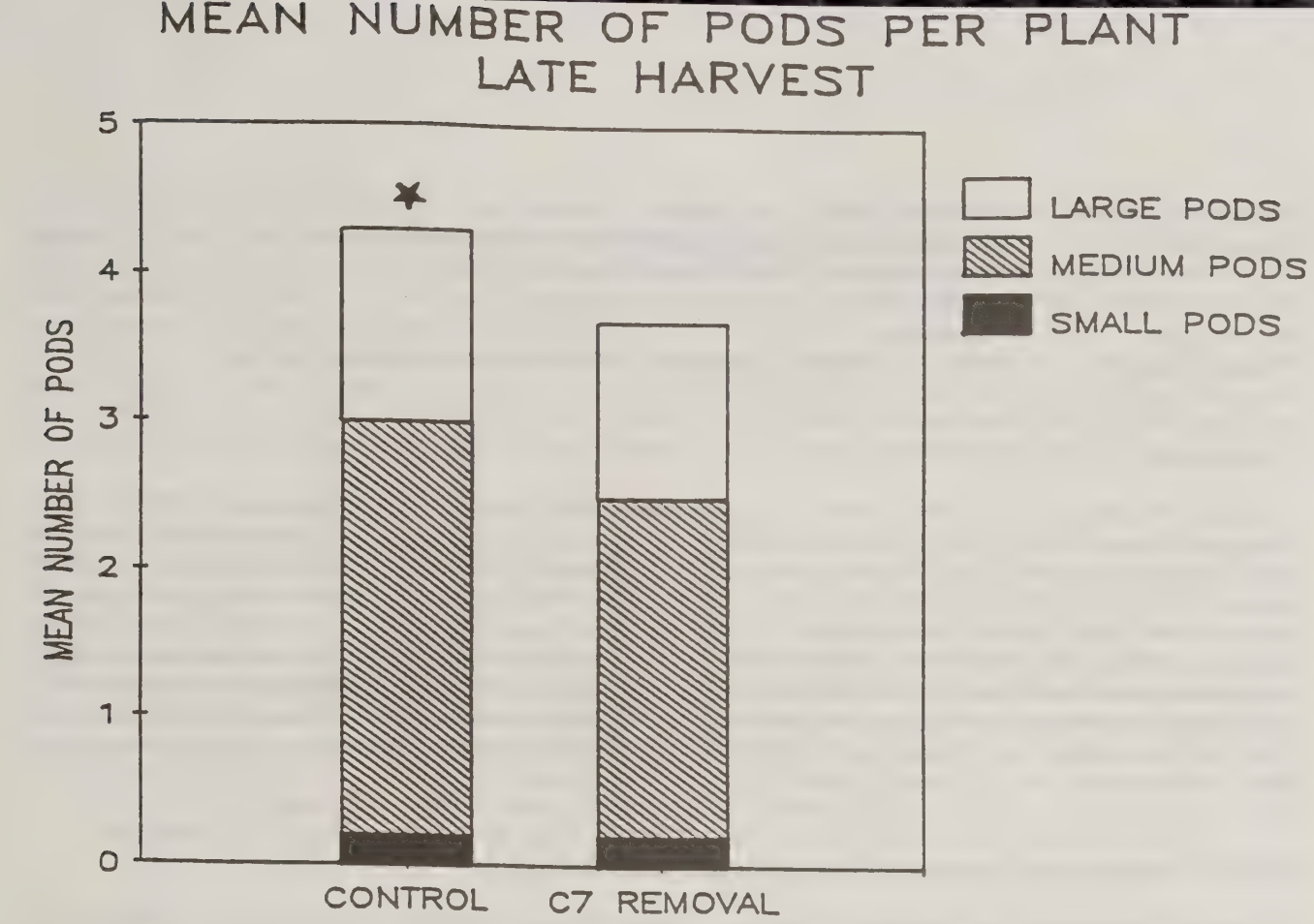


Fig. 3. Mean number of small, medium and large pods at late-season harvest; * indicates significance ($P<0.05$) between treatments.

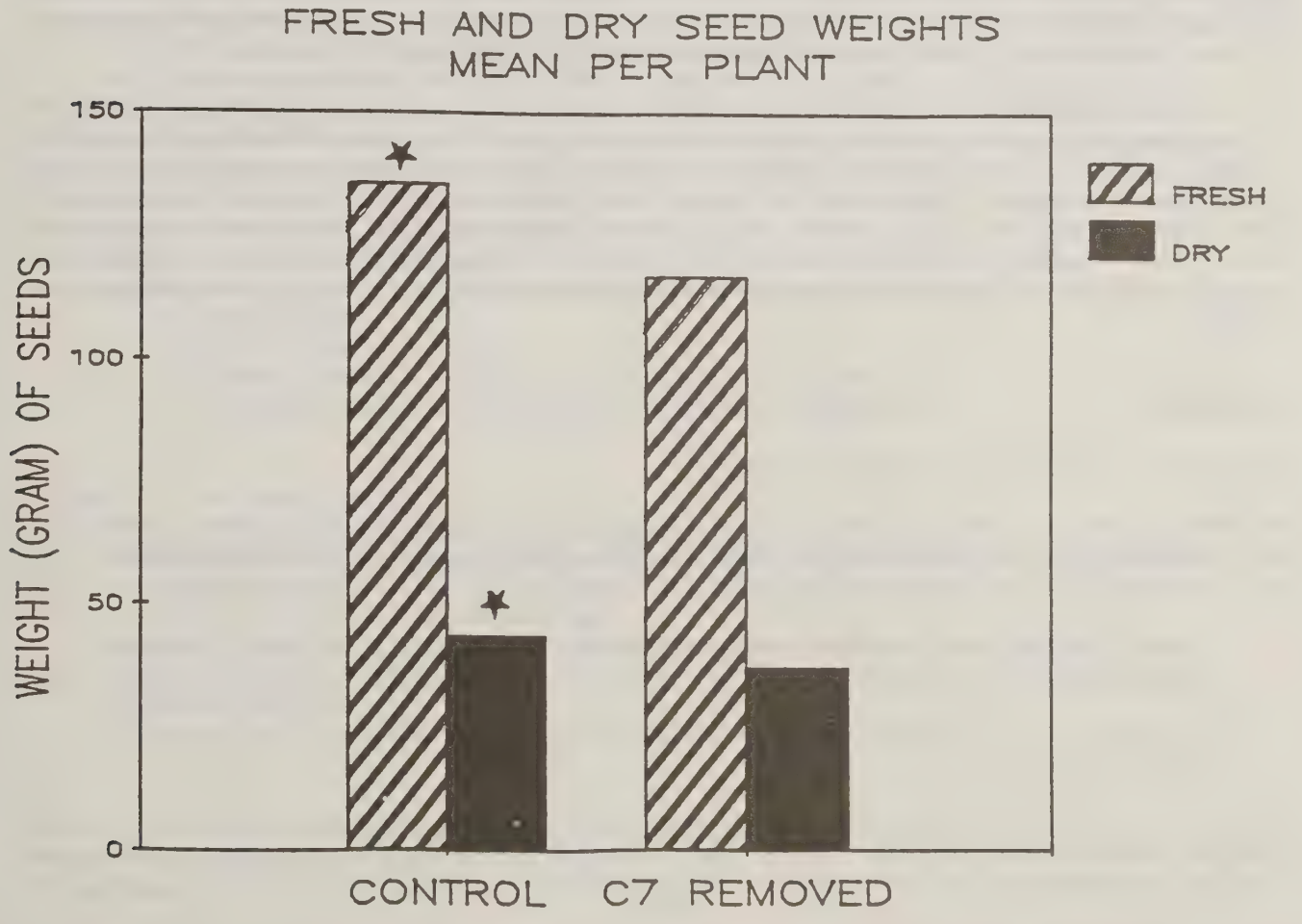


Fig. 4. Mean fresh and dry weights of seeds at late-season harvest; * indicates significance ($P<0.05$) between treatments.

Project Number:	ABC 89.1.1
Project Title:	Development and Validation of Field Sampling Methods for Russian Wheat Aphid Biological Control Survey
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Preliminary
Project Leader:	William C. Kauffman, assisted by Kim Murchison and Bob Tardif

Introduction

The Otis Methods Development Center has supported Plant Protection and Quarantine's (PPQ) Aphid Biological Control (ABC) Project since 1987 with sole responsibility for evaluating the effectiveness of natural enemies against target aphids. This important role in evaluation is continued by Otis Methods Development Center in the biological control of the Russian wheat aphid (RWA), a recent (1986) invading pest of small grains in the United States. The following protocol was developed to guide field sampling of RWA and its natural enemies, both native and exotic. This plan is intended to improve and standardize sampling methodology which will provide relative estimates of pest aphid densities. This information will comprise a qualitative evaluation of beneficial organisms. When combined with more quantitative measurements, these data will thus enable comprehensive evaluation of the impact of predators, parasitoids and pathogens on RWA populations.

Russian Wheat Aphid Biological Control Survey Methodology

USDA-APHIS undertook in 1988 a new initiative in classical biocontrol of the Russian wheat aphid (RWA), *Diuraphis noxia*, through foreign exploration for natural enemies. Several exotic species of parasitoids and predators have already been introduced through quarantine and field released as a results of these cooperative efforts of USDA-APHIS, the CIBC, British Museum, USDA-ARS and university/state scientists. Selected beneficial species which show promise for RWA suppression are now being cultured by two USDA-APHIS Biological Control Laboratories for mass rearing in support of field releases.

It is anticipated that establishment and colonization of these introduced species will augment present levels of parasitism and predation of aphid populations in small grains. This enhancement of existing natural enemies is hoped to lower the relative population densities of RWA throughout it geographic range in the United States. Therefore, a region-wide field survey of RWA densities and natural enemies associated with RWA in small grains is needed in order to measure the agricultural benefits of this investment in biological control. This concept of a systematic survey coordinated by APHIS has been enthusiastically supported by states involved, and the methodology has been refined following preliminary testing in a few selected states in 1988.

Objectives

The objective of this regional biocontrol survey is:

Provide information on RWA and natural enemies before and after releases of exotic natural enemies and to assess the impact of these releases on RWA and other aphids in small grains. Specifically, the survey will:

1) Identify the species and abundance of natural enemies, both indigenous and exotic, that exist in RWA-infested fields.

2) Estimate relative population densities of RWA and cohabiting aphids in small grain fields infested with RWA.

Aphid and beneficial arthropod data will be gathered by a combination of chemical extraction and visual census of plant tillers and by sweep netting. These varied sampling techniques will minimize time spent in the field and increase accuracy of the survey. Sorting and species identification of collected specimens will

be handled by APHIS laboratories. Instructional materials and collecting equipment will be supplied as needed by the APHIS Otis Methods Development Center. The survey will, ideally, involve 4-15 fields in all RWA states (presently 15) through the growing season. These fields will be selected based on proximity to field releases of natural enemies and, when possible, should be in conjunction with fields used in the suction trap network. USDA-APHIS had hoped to initiate this comprehensive survey in 1989. However, the complexity of the activities and the need for significant back-up lab support to process samples and some uncertainty as to parasite taxonomy, prompts us to defer large-scale implementation of this survey until 1990. Thus, any field activities in 1989 will be directed towards perfecting the methods that will be used in the survey. The outlook for adequate funding in 1990 to perform this important survey is very good.

Materials and Methods

A. Field Selection

1. The survey coordinator in each state should select wheat fields with moderate to heavy infestations of RWA for this multi-state survey. Within a "generally infested" area, choose 2-5 fields within a 10-20 miles radius; this constitutes one geographic location. Fields in a geographic location must be at lease 25-50 miles from releases of exotic natural enemies prior to and during the first two years of the survey (1990-1991), thus providing baseline data. Niles Biocontrol Laboratory is the current repository for these release records. Releases of beneficial species (coordinated by APHIS) will be conducted in the vicinity of these survey fields so to create an "after release" situation for comparison.

2. When possible, repeat this field selection in 2-3 geographic locations which are at least 50 miles apart and preferably on a north-south gradient throughout the state.

3. Winter wheat fields should be selected whenever possible; however, spring wheat may be substituted if it is the primary wheat crop in the state. The criterion for candidate fields should be an existing population of RWA in that field (fall or early spring determination). Such fields may be accompanied by the typical RWA symptoms (curling, pink/purple leaf blotching or streaking often bordered by white). Select fields of growers who are less likely to apply pesticides for RWA control. Field statistics, pesticide usage and the occurrence of grazing by cattle should be recorded on page one of the survey forms.

4. Selected fields are to be surveyed as follows:

   a) **every two weeks** throughout the growing season, weather permitting, from tillering through harvest,
   b) between 8:00 AM and 12 noon,
   c) when the temperature is above 45˚F (exceptions in winter time),
   d) after the prescribed waiting period following pesticide application.

B. How to Survey within the Field

1. Select 10 sampling sites arranged diagonally in the field. Walk 20 paces into the field for the first site, 20 paces further for the second site, etc., for a total of 10 sites. The sites selected in the field should differ from week to week.

2. At each site, take the following four distinct types of samples in the sequence indicated for insect and crop data:

a) **Tillers for Chemical Extraction**

(Aphids, larval and adult predators)

Using a scissors, cut at the base 10 tillers (= stems originating at crown of plant) at random within a 10-foot radius. After cutting the first tiller, immediately transfer it to a 1-gallon plastic ziplock bag in such a way that disturbance and escape of insects is minimized. For example, tillers longer than 12 inches can be inserted into the bag while cutting off the tiller. Follow this procedure until all 10 tillers are collected in the bag, then affix a gummed label (label A 1-10, indicating date, field, field site, etc.). Accumulate these 10 plastic bags (1 per site) in a large paper bag, then place the bag into an ice chest to prevent overheating until processing.

Insects should be extracted as soon as possible at the field edge after all sampling (#2a-d) is completed. If extraction is not possible at the field because of inclement conditions (i.e., windy, precipitation, etc.), this process should be done immediately back at laboratory/station/office. Use the chemical extraction can (specified below) in an open, well-ventilated location protected from the wind or under an exhaust hood. Care should be taken to prevent inhalation of this chemical or contact with one's skin.

Chemical Extraction Can - Apparatus and Methodology:

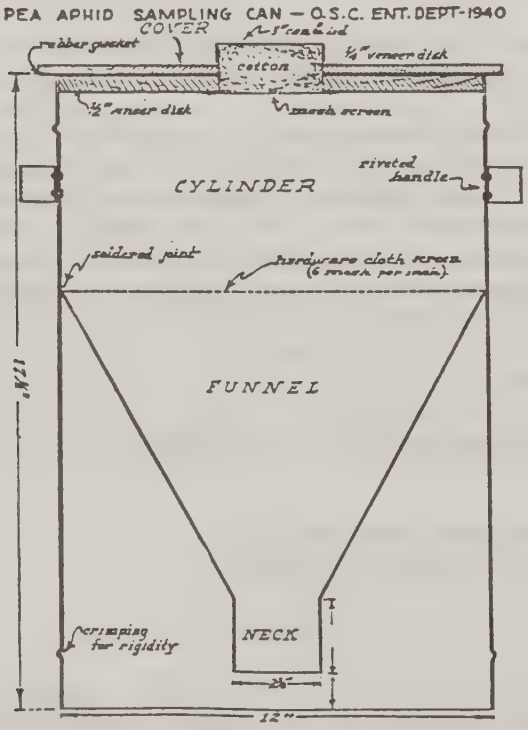


FIG. 2.—Diagram of can showing details of construction.

Reference:

Gray, K. W. and J. Schuh, 1941. J. Econ. Entomol. 34:411-415

These extraction cans will be constructed as units consisting of 10 extraction cans (i.e., need one 10-can unit to sample a field) and will be supplied by Otis Methods Development Center upon request from participating states.

Procedure for extracting arthropods:

1) Saturate the cotton wicks located in extraction can lids with 4-Methyl-2-Pentanone ($CH_3COCH_2CH_2$; Methyl isobutyl ketone; a flammable, toxic irritant).

Attach glass collection vials containing 70% ethanol (EtOH) to the bottom of the funnel.

2) Dump wheat tillers and their contents from each field site into an extraction can and replace the can lid. Transfer "A" labels from ziplock bags to corresponding vials.

3) After 5 minutes, gently shake each can 10 times so that the most active insects fall into the alcohol vial. Remove the lid, then proceed to examine tillers for mummified and diseased aphids, as directed in Part B below.

4) Now replace lids and vigorously shake the cans 50 times to complete the dislodgement of aphids and predators.

5) Species and abundance of RWA and other aphids, small predators and larval stages of predators (especially coccinellids) will be determined at the Niles Biocontrol Laboratory.

b) **Tillers for Removal of Mummies and Diseased Aphids**

Carefully examine (one by one) each of the 10 tillers used in the chemical extraction process (above) in search of mummified or diseased aphids. These aphids are uncharacteristically brown or black, are immobile and remain attached to the leaves. Visually search the tillers, unrolling any damaged by RWA, directly over the open extraction can to catch any insects which drop from the tiller. With small dissecting scissors, cut away the leaf from around the mummy or diseased aphid and place mummies into gelatin capsules (preferably #00, 0 or smaller) with <u>one</u> mummy or diseased aphid per gelatin capsule. Combine all gelatin capsules from a particular site in the field into a labeled container (label B 1-10) held at room temperature. This visual examination may be done at the field or immediately upon returning to the laboratory, to preempt emergence of adults of parasitic species. From these collections, rates of parasitism by species and pathogens will be determined for aphid hosts in the sample (Niles Laboratory).

c) **Sweep Net Sample**

At each field site, in areas not disturbed by tiller sampling, take one sweep net sample consisting of 5 sweeps (180° sweep arc) with a standard 15-inch muslin sweep net. Empty the sweep net contents into a 1 gallon plastic ziplock bag (label C 1-10). Transport bags from the field in an ice chest. Immediately freeze bags, then transfer the contents of each bag to a glass vial containing 70% ethanol (again, label C-10). The target insects in this sample are primarily lady beetle adults and larvae, but also nabids, lacewings, etc.

d) **Plant Stage and Tiller Density**

At each of the 10 sites in the field, use a throwstick to randomly choose two 1-foot sections of row and record the following data:

1) Count the number of tillers in each 1-foot row sample, then use Appendix A to calculate average number of tillers on a per square foot basis.

2) Record the overall stage of plant development in the 1-foot row sample using Zadok's scale (Appendix B).

Combine the following samples from a single field in a **sealed** shipping container:

- 10 chemical extraction can samples (labels A 1-10)
- 10 sets of mummies and diseased aphids enclosed in gelatine capsules (labels B 1-10)
- 10 sweep net samples (labels C 1-10)

(Note that A-C represents sample type and 1-10 represents field site)

and send to:

Robert V. Flanders
USDA, APHIS, PPQ
Biological Control Laboratory
2534 South 11th Street
Niles, MI 49120
(616) 683-3563; (FTS) 333-8212

Insect counts and species identification data compiled at the Niles Laboratory will be sent by Niles to the individual state as soon as possible. It is then the responsibility of each state to enter this sampling data into the NAPIS database promptly upon receiving the laboratory diagnostic report from the Niles Laboratory.

The Niles Laboratory will also send a photocopy of this diagnostic report for survey analysis to:

William C. Kauffman
USDA, APHIS, SCI & TECH
Otis Methods Development Center
Building 1398
Otis ANGB, MA 02542-5008
(508) 563-9303; (FTS) 828-9355

Coordination for this RWA Biological Control Survey will be a cooperative effort between Otis Methods Development Center and Niles Biocontrol Laboratory.

APPENDIX A: CALCULATING TILLERS PER SQUARE FOOT FROM TILLERS IN A 1-FOOT SECTION OF ROW AND THE ROW WIDTH.

Multiply the appropriate factor (corresponding to row sparing) times the average number of tillers counted at each of 10 sites. Record these tiller densities in the space provided on the forms.

Row spacing, inches	Multiplication factor
2	6.0
4	3.0
6	2.0
8	1.33
10	1.2
12	1.0
14	0.86
16	0.75
18	0.67
20	0.60

APPENDIX B: ZADOK'S ONE DIGIT CODE FOR PRINCIPLE GROWTH STAGES OF CEREALS

Code	Growth Stage Description	
(0	Germination	) Stages prior to data recording
(1	Seedling growth)	
2	Tillering	
3	Stem elongation, including flag leaf appearance	
4	Booting	
5	Inflorescence emergence	
6	Anthesis (blooming)	
7	Milk development	
8	Dough development	
9	Ripening	

Field Validation

Thirty chemical extraction funnels were built at the Otis Methods Development Center (designed by Bill Kauffman and Bob Tardif) for use in testing this sampling technique for RWA field populations. Winter wheat fields were sampled during May 1-12 at the following locations: four fields in western Kansas (Wallace and Logan Counties); two fields in Oklahoma (Beckham County); and one field in New Mexico (Curry County. A photographer arranged through APHIS-Media Services was on site one day to shoot footage of our sampling methodology. The intended use of the film was to produce to 15-minute videotape that would accompany a survey manual for training APHIS and state personnel in the sampling of RWA and beneficial species.

A conclusion from this preliminary field sampling was that the chemical extraction funnel, although satisfactory for removing RWA from exposed parts of the plant, was inadequate for extracting RWA from tightly rolled leaves and from grain heads. In these latter two situations it was necessary, prior to placing the tillers in the chemical extraction funnel, to unroll the leaves, brush them with a camel hair brush and macerate the grain heads. By these actions, RWA feeding would be interrupted and the vapors would permeate these protected feeding sites on the plant, resulting in efficient extraction of RWA. However, the large of amount of time involved in this hand manipulation of the tillers and the requirement that this work be done in an area protected from wind and precipitation (i.e., often away from the field site) severely limits the usefulness of this chemical extraction funnel in field sampling of RWA. In addition, vapors of 4-methyl-2-pentanone are very pervasive, long-lasting and noxious even in a well-ventilated area. Possibly, careful removal of RWA with a camel hair brush into a white enamel pan would be a useful alternative to this chemical extraction method. Otherwise, the methods outlined for collecting tillers for aphid and mummy determinations, the method of sweep netting predatory arthropods, and procedures for isolating and shipping insects to the Niles Laboratory were highly suitable.

Epilogue

After much discussion among APHIS personnel involved in the Aphid Biological Control (ABC) Project, it was agreed that evaluation of RWA biological control should involve three parts: program evaluation, biological evaluation and economic evaluation. These three areas, although operating under somewhat different goals, are interrelated and significant progress in ABC evaluation will rely on dedicated cooperation among all laboratories and individuals involved.

Program Evaluation will be coordinated by Bob Flanders (Niles Laboratory) as the major thrust of their natural enemy establishment survey. It was agreed that this establishment survey will address the primary objectives of the proposed RWA Biological Control Survey; therefore, this survey proposed by Otis Methods Development Center will be incorporated into the Niles Laboratory's work plan for surveying field populations of RWA for program evaluation. The aim of pre-release, release, and post-release surveys at predator and parasitoid release sites in all RWA-infested states is to determine recovery and establishment success of introduced entomophagous species. By incorporating percent parasitism and abundance of predators with host/prey densities, the survey will attempt to determine general population changes of RWA as the result of implementation of biological control.

Biological Evaluation will be coordinated by Bill Kauffman (Otis Methods Development Center), and will involve cooperative research agreements established with university or state scientists. The objectives of this research are to assess, through highly quantitative field experimentation, the impact of predators and parasitoids (possibly including pathogens) on RWA and cohabiting aphids in small grains, and, furthermore, to determine the level of crop protection afforded by implementation of classical biological control of RWA. These objectives will likely be achieved through the use of cages (e.g., exclusion, inclusion or interference/partial exclusion types) or insecticidal exclusion methods to contrast aphid densities before/after introductions and in the presence/absence of natural enemies. However, biological evaluation of the impact of beneficial species of RWA must be preceded by establishment of baseline data for RWA and indigenous species at these field sites as well as refining field evaluation techniques. Intensive determination of aphid densities and incidence of parasitization and predation will establish the level of aphid suppression directly attributed to the action of parasitic and predatory species. Other parameters (biotic or abiotic) which are

important in influencing the predator-prey or parasitoid-host relationship may also be investigated. Crop responses to aphid pressure and yield data will be an integral aspect of this field study.

A RWA Biological Control Evaluation Team can be assembled by early 1990 and will include cooperators, research consultants and USDA administrators. The Evaluation Team will meet annually to review progress of the project and to plan future activities.

Economic Evaluation, the final phase of the evaluation project, will be coordinated by Phil Kingsley (Otis Methods Development Center) and will involve an agricultural economist(s) familiar with small grains economics. The economic analysis will attempt to relate field augmentation of beneficial insects and the expected decline in RWA densities in terms of savings to the grower, the agricultural community in general, and the consumer sector. Changes in crop yields and pesticide usage will be the driving force in the economic models. For example, a typical cost-benefit analysis may be compared with an econometric-simulation model. Baseline economic data for the crop will be compared to crop economics following full-scale implementation of RWA biocontrol. It is recommended that this economist be involved from the initial planning phase to advise how this Evaluation Team can best provide data needed for economic analysis while at the same time gathering data for biological and program evaluation.

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leader: Philip C. Kingsley

This was the final year of data collection for the Alfalfa Weevil Evaluation Project. We would like to take this opportunity to thank all those participating, including field surveyors, their supervisors, and laboratory technicians. Their excellent work has been a major contributing factor to the success of this project. What follows, for our final laboratory report, are some excerpts from the first draft of a future publication authored by P. C. Kingsley, M. Bryan, W. H. Day, T. L. Burger, R. J Dysart and C. P. Schwalbe.

Program

In 1979 and 1980, the Alfalfa Weevil Parasitoid Redistribution Program was developed cooperatively with two USDA Services (ARS, APHIS) and State Departments of Agriculture. The major objective of this program was to distribute and establish two major parasitoid species, already established in the northeast, across the country. This objective would be accomplished in three phases, Detection (pre-release) Survey, Collection and Redistribution, and Establishment (post-release) Survey, and was coordinated from APHIS's Niles Biological Control Laboratory in Niles, Michigan. In addition, the program planners recognized a unique opportunity to evaluate the long term benefits derived from this large scale biological control program. A concurrent evaluation project was designed, therefore, to meet this objective and was coordinated from APHIS's Otis Methods Development Center located on Cape Cod, Massachusetts.

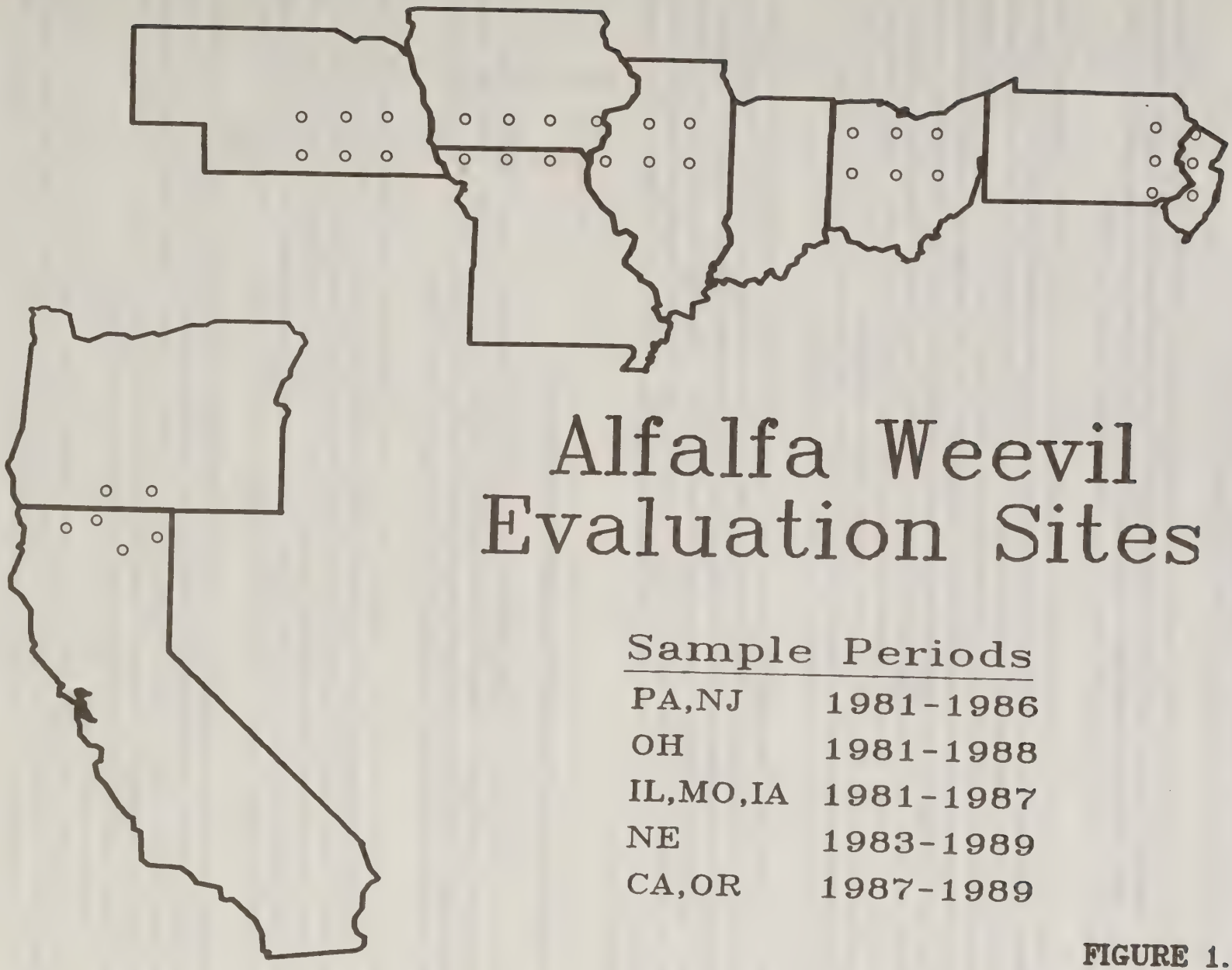
Evaluation

The evaluation survey was initiated in 1981, when one hundred and twenty fields were surveyed in four areas (I-IV) located in six states (Figure 1). A fifth area (O) was added in 1983. Within each area, six sites (counties) were selected on approximately 65 mile centers and five fields were sampled per site resulting in 30 fields per area. Areas were located on a latitudinal transect far enough north to avoid the complication of fall laid eggs. Each field was sampled nine times at weekly intervals during the first and early second cuttings. Sweep nets were used to estimate the population densities of alfalfa weevil adults and larvae (#/100 sweeps). In addition, up to 300 larvae and 100 adults were sent to laboratories for dissection or rearing, to determine parasitism rates and the identity of established parasitoid species. Alfalfa weevil adults and larvae were dissected by the personnel at the Niles Biological Control Laboratory and parasitoids were reared at Niles and three other processing laboratories; Kansas State Department of Agriculture in Topeka, Colorado State Department of Plant and Industry at Palisades, and at APHIS's Otis Methods Development Center on Cape Cod, Massachusetts.

Each year, in cooperation with the Economic Research Service, participating growers were surveyed for information pertaining to the economics of growing alfalfa on their farm. We were interested in documenting trends in the use of insecticides as well as changes in their attitudes towards alfalfa pest control. Data collected included acreage, yields, and inputs such fertilizer and pesticides.

Parasitoids

Parasitism and establishment data were collected for the three most important alfalfa weevil parasitoids; two of the larval stage (*Bathyplectes anurus* [Thomson] and *B. curculionis* [Thomson]), and one of the adult stage (*Microctonus aethiopoides* Loan).



Alfalfa Weevil Evaluation Sites

Sample Periods	
PA,NJ	1981-1986
OH	1981-1988
IL,MO,IA	1981-1987
NE	1983-1989
CA,OR	1987-1989

FIGURE 1.

Bathyplectes curculionis (Thomson) (Hymenoptera:Ichneumonidae)

Bathyplectes curculionis was the first parasitoid to be introduced from Europe in 1911 (Chamberlin 1926). With its strong natural dispersal ability, and help from biological control workers in the past (see Hagen et al., 1976), its early distribution was virtually the same as that of the alfalfa weevil. *Bathyplectes curculionis* was recovered in 98 percent of 437 counties sampled during our detection survey. For this reason, *B. curculionis* was not a target species in the redistribution program.

Adults emerge from over wintering cocoons in the spring and prefer to parasitize early instars of the alfalfa weevil. After 218 C degree days (Eklund and Simpson, 1977) the parasite emerges and spins a dark brown cocoon, often inside the weevils own netlike cocoon. A small percentage of nondiapausing individuals construct light brown cocoons and emerge to produce a partial second generation.

Several factors seem to limit the impact of *B. curculionis* on alfalfa weevil populations in the eastern United States. Peak parasitism is not well synchronized with its host and generally occurs after the maximum densities of alfalfa weevil larvae (Chamberlin, 1926) (Figure 2). This species is also routinely encapsulated by eastern strain weevils (van den Bosch and Dietrick, 1959; Puttler, 1967, 1974). When a single parasite egg is laid per host, effective parasitism can be reduced from 20 to 45 percent by encapsulation (Berberet et al 1987). Nonfunctional ovaries are also a factor in limiting this parasitoids effectiveness in the east (Dowell and Horn, 1977; Yeargan and Pass, 1978; Day, 1983). Day (1983), for example, found that 10% of all *B. curculionis* females collected from four eastern states were infertile. Several species of hyperparasitoids have also been reported from *B. curculionis* (Puttler, 1966; Pike and Burkhardt, 1974; Rethwisch and Manglitz, 1986). Simpson et al (1979) reported that over a five year period in Colorado, 53% of overwintering cocoons were killed by five species of hyperparasitoids.

Effective generational parasitism (Southwood and Jepson, 1962) in our evaluation sites averaged 16.5% (sd = 12.71 N = 133 site years 1) when not in competition with its congener *B. anurus.* It should be noted, however, that the western strain of the alfalfa weevil does not have the ability to encapsulate *B. curculionis* (van den Bosch and Dietrick, 1959), as does the eastern strain. This differential immunological response may be reflected by higher parasitism rates in the west. In California and Oregon for example, parasitism was 38.7 (sd = 31.37 N = 12 site years) in 30 fields surveyed in six sites over the 1987 and 1988 seasons (unpublished pck).

Bathyplectes anurus (Thomson) (Hymenoptera:Ichneumonidae)

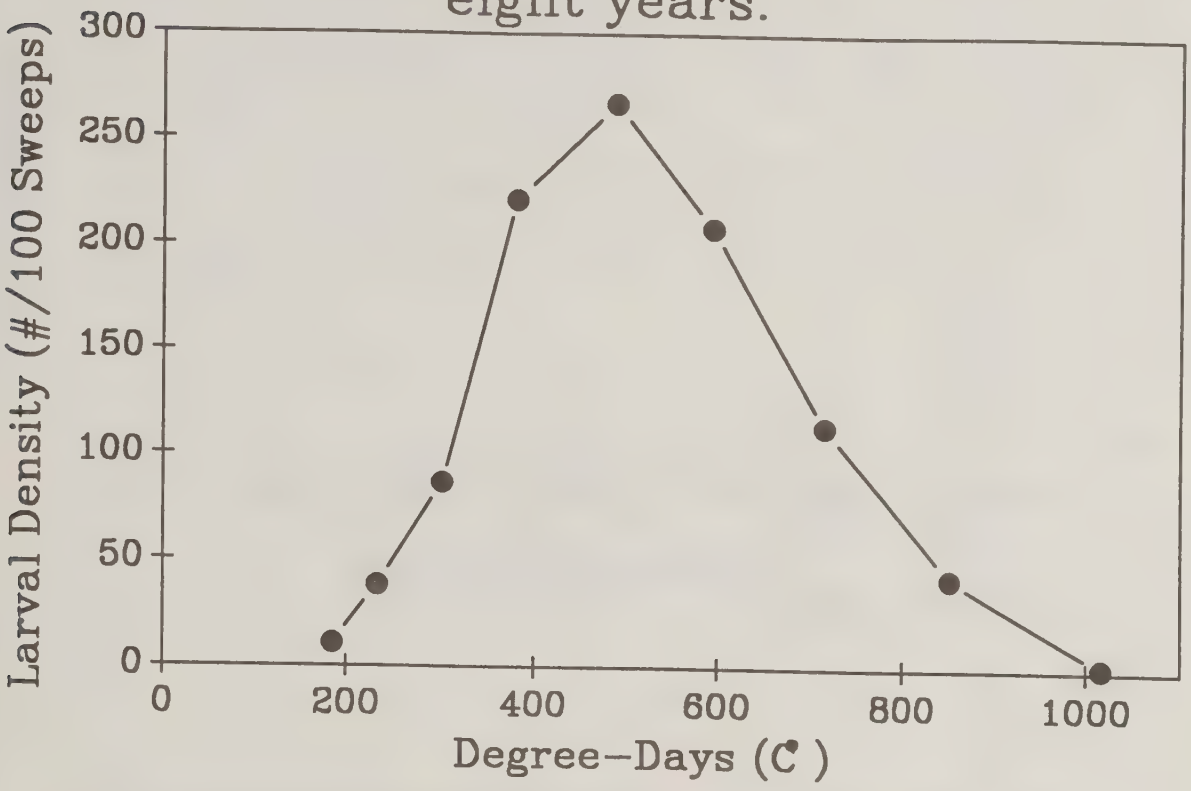
The biology of *B. anurus* is similar to that of its congener *B. curculionis,* except that no partial second generation is produced. It was first introduced from Europe into the northeast in 1960 and was well established in Pennsylvania and New Jersey by 1966 (Dysart and Day, 1976). Fifteen years later, detection surveys found its distribution still limited to the Northeastern United States.

Nearly 15 million *B. anurus* were released, either as larvae in parasitized alfalfa weevil larvae, or as adult parasitoids from insectories, in 544 counties in 38 states during the collection and distribution phase of this program. Overall positive recovery rates were 45%, resulting in 245 new county records (Table 1). As expected, establishment rates are closely related to both the number of parasitoids released and the number of hosts collected during the recovery survey. For *B. anurus,* higher recovery rates (60%) were attained when optimal numbers were released (Figure 3) and a minimum of 1000 hosts were collected (Figure 4).

Bathyplectes anurus was released in ten states (47 counties) where the majority of alfalfa weevils collected have been identified as western strain (Donald Vacek, personal communication). Recovery rates were similar in these counties (45%), when compared to 497 counties in eastern strain states (42%), indicating a compatibility between *B. anurus* and the western strain weevils (Table 1). This is not the case, however, with the parasitoid of the adult, *Microctonus aethiopoides* (see below).

The westward movement of *B. anurus* has been documented since 1981 as part of the evaluation survey. Establishment, measured as percent field recovery, has increased consistently in Missouri/Iowa, Illinois and Ohio. For example, in 1981 we failed to collect this species in any of the thirty fields surveyed in Illinois

Generalized phenology of alfalfa weevil larvae and the number parasitized, based on 30 alfalfa fields sampled in Ohio for eight years.



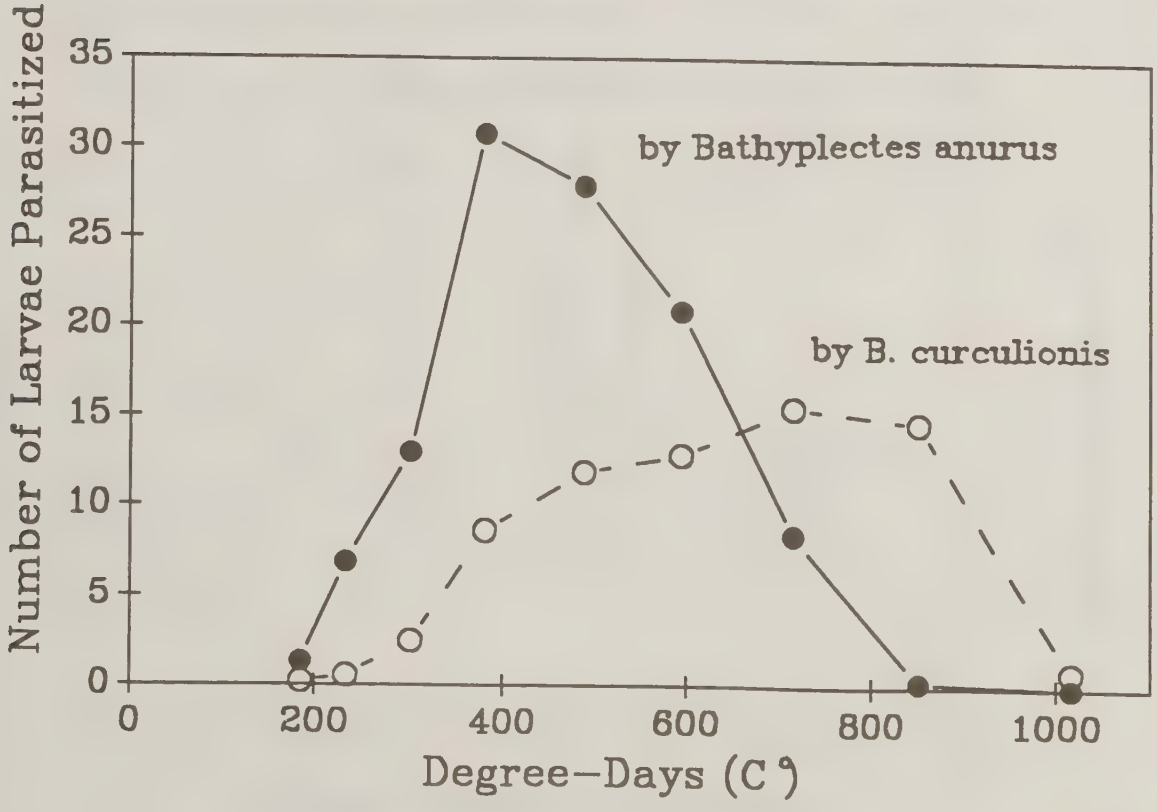


FIGURE 2.

AW Parasitoid Establishment Rates.

	Bathyplectes anurus	*Microctonus aethiopoides*
Overall	45.0% (245/544)	37.0% (78/211)
by AW Strain		
>50% Eastern	45.1% (224/497)	42.9% (73/170)
>50% Western	44.6% (21/47)	12.2% (5/41)

TABLE 1.

Bathyplectes anurus recovery rates by the number released.

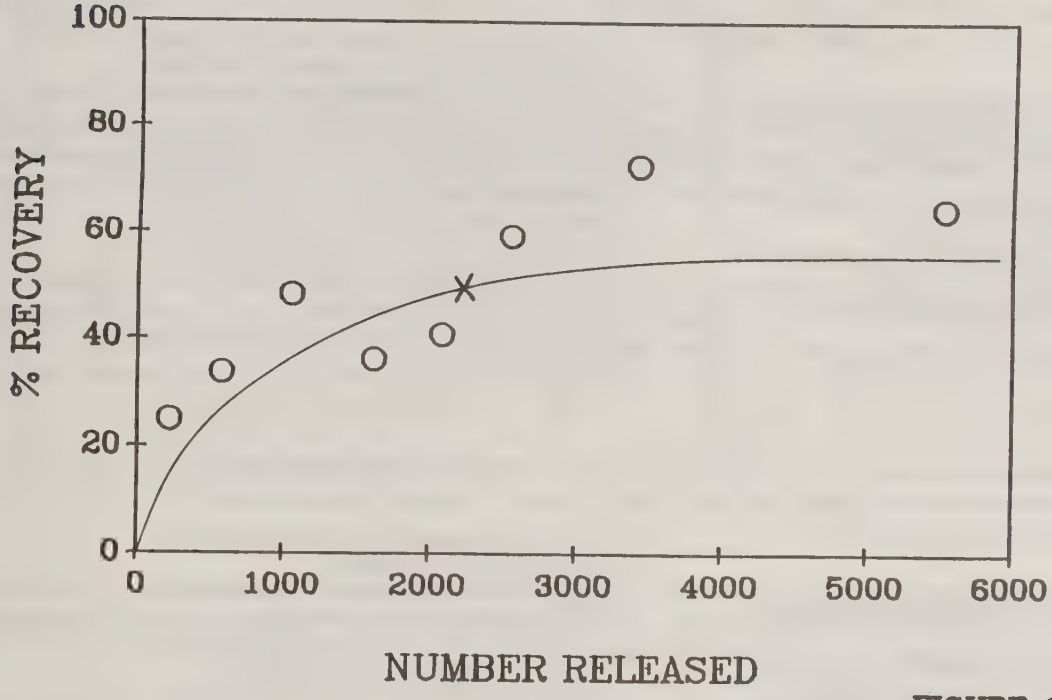


FIGURE 3.

Bathyplectes anurus recovery rates by the number of hosts collected.

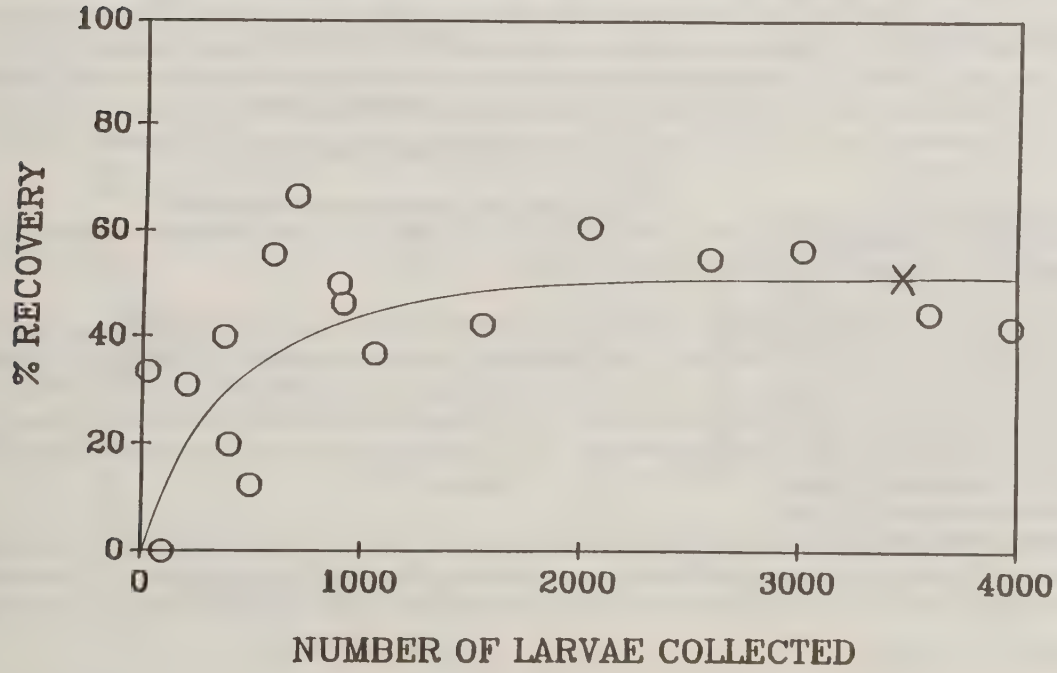


FIGURE 4.

(Figure 5). By 1986, however, this species was recovered in 70 percent of these fields. Although lagging behind slightly, parasitism by *B. anurus* also showed a concomitant increase with establishment (Figure 6).

Certainly, a portion of this movement west was due to natural emigration from previous releases, especially in Ohio where *B. anurus* was initially released in 1965 and was recovered for the first time in 1972.

Dowell (1977) predicted that *B. anurus* would become the dominant member of the larval parasitoid guild when sympatric with *B. curculionis* in the east, and cited several reasons for this competitive advantage (see also Yeargan et al., 1978). These include: better synchronization with its host (Figure 2), high reproductive capacity, faster search and handling times, low rates of encapsulation (see Puttler 1967), and the earlier elimination of supernumeraries.

This shift in dominance was documented during the evaluation survey in Ohio. Field recovery rates (establishment), as mentioned above, increased consistently from less than 40 percent of the thirty fields in 1981, to 85 percent in 1986 (Figure 5). During this period, the competitive displacement of *B. curculionis* is indicated by a reduction in its portion of the overall larval parasitism inflicted by both species, from 83 to 34 percent (Figure 7).

Data from 30 evaluation survey fields located in Pennsylvania and New Jersey show the culmination of this trend for B. anurus dominance (also see Day, 1983). Here, *B. anurus* has accounted for over 93% of the total larval parasitism since 1981.

Larval parasitism has been enhanced in Ohio with the increasing importance of *B. anurus.* This becomes clear when fields are grouped by the dominant larval parasitoid (Table 2). Where *B. anurus* accounts for greater than 50% of the larval mortality, overall parasitism rates are significantly higher.

Through life table and laboratory studies, *B. anurus* has been shown to be independent of its host density (Latheef et al., 1977; Dowell, 1977; Harcourt et al., 1977). That is, this species does not act in a way that would stabilize alfalfa weevil populations from generation to generation. Although *B. anurus* may not, by itself, regulate alfalfa weevil populations, this species can impart significant mortality, especially in years where disease rates are low. Both species of *Bathyplectes* can also inflict significant incidental mortality due to oviposition trauma etc. (Latheef et al., 1979).

Microctonus aethiopoides Loan (Hymenoptera:Braconidae)

Microctonus aethiopoides, a parasitoid of the adult stage, was initially released against the alfalfa weevil in New Jersey in 1957. By 1961 it had become firmly established in the eastern United States (Day et al., 1971). *Microctonus aethiopoides* is bivoltine and overwinters as a first instar larva inside overwintering adult alfalfa weevils. Adult wasps emerge in the early spring and oviposit in surviving unparasitized spring weevils. The resulting adult wasps from this non diapausing generation then lay eggs in newly eclosed weevil adults that will ultimately overwinter, harboring first instar *M. aethiopoides* (see Loan and Holdaway, 1961; Van Driesche and Gyrisco, 1979).

At the initiation of the Alfalfa Weevil Biological Control program in 1980, the detection survey indicated that *M. aethiopoides* was already established in 19 states (Day et al, 1971).

During the first year of the evaluation survey in 1981, we recovered this species from 100% of the sixty fields surveyed in Ohio and Illinois and 40% of the thirty fields surveyed in Missouri and Iowa (Figure 5). Nebraska was first surveyed in 1983 and *M. aethiopoides* occurred in only one of the thirty alfalfa fields sampled. By 1988, 490 thousand *M. aethiopoides* had been released at 211 counties in 34 states, including 11.8 and 33.8 thousand in Missouri/Iowa and Nebraska respectively. By 1984 this species was recovered from all the Missouri and Iowa fields, and by 1988, from 76.7% of the Nebraska fields.

Following establishment in Missouri and Iowa, there was a steady increase in parasitism rates from 2.3% in 1981 to 40% in 1985 (Figure 8). Nebraska alfalfa fields are 6-7 years behind Missouri and Iowa, in terms of establishment and parasitism, but have also shown an increase from .04% in 1983 to 4.4% in 1988 (Figure 8).

Percentage of fields (N=30) where three parasite species were recovered.

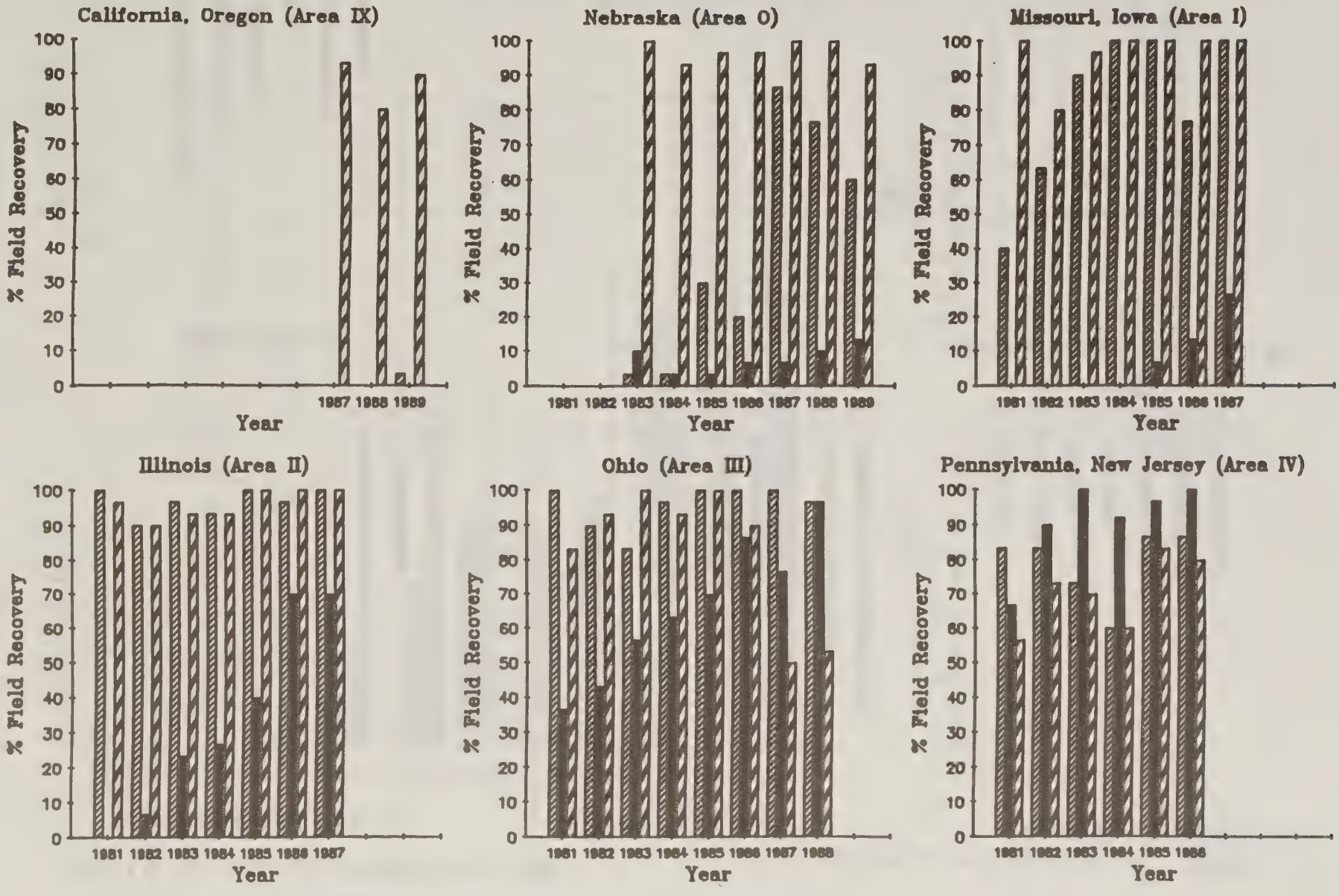


FIGURE 5.

PERCENT PARASITISM BY *Bathyplectes* sp. OF AW LARVAE BY AREA AND YEAR.

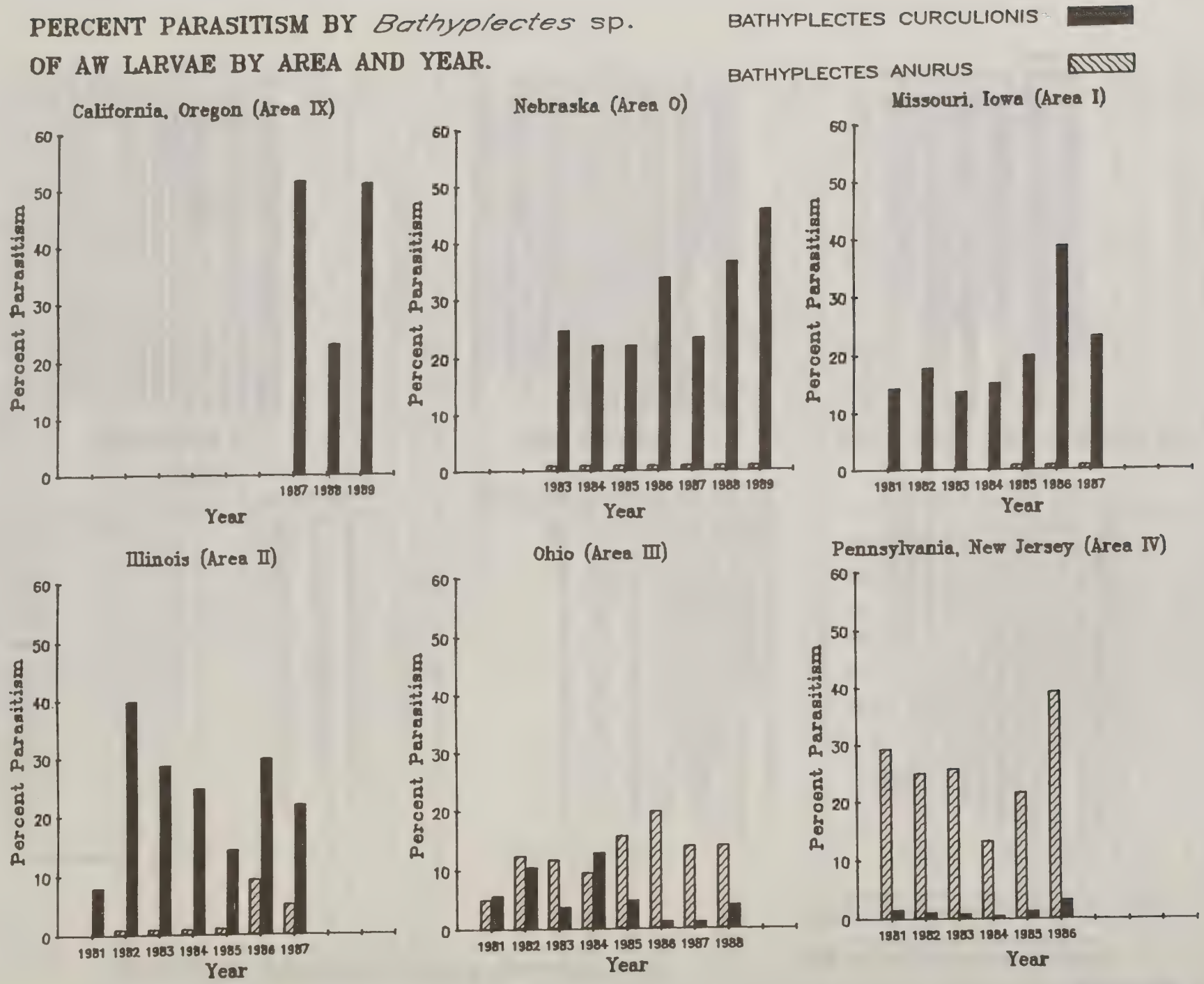


FIGURE 6.

Relative percent parasitism of two alfalfa weevil larval parasites in 30 Ohio alfalfa fields.

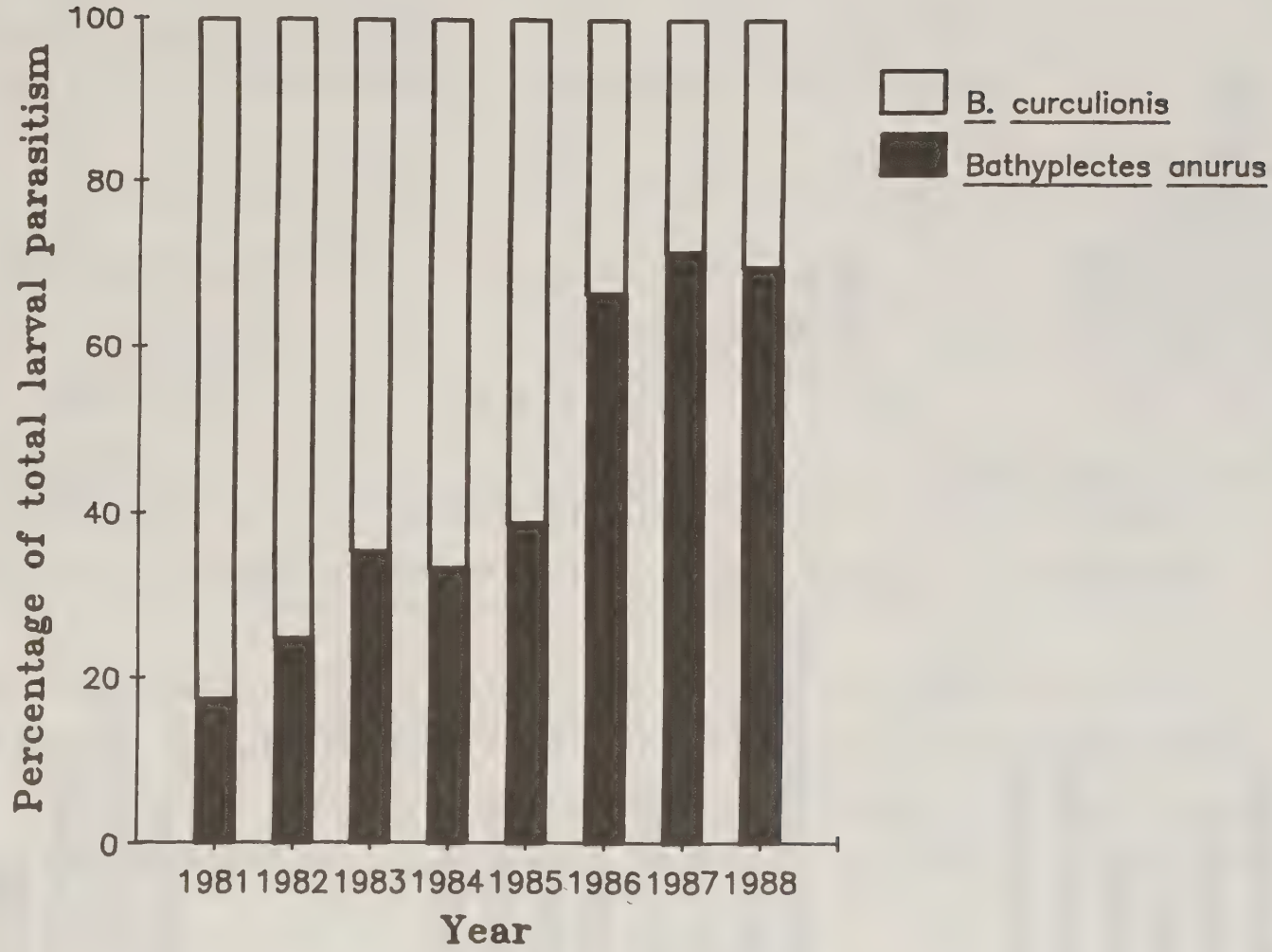


FIGURE 7.

Overall parasitism of alfalfa weevil (Hypera postica) larvae in Ohio alfalfa fields where each of the two congeners, Bathyplectes anurus and B. curculionis, are dominant in terms of relative parasitism.

Dominant Larval Parasitoid *	Overall Larval Parasitism	SD	Number of Fields **
B. anurus	24.9%	17.59	103
B. curculionis	13.9%	9.81	137

P < .0001 T-Test

* Accounts for > 50% of the total larval parasitism.

** 30 fields/year x 8 years

TABLE 2.

PERCENT PARASITISM BY *Microctonus aethiopoides* OF AW ADULTS BY AREA AND YEAR.

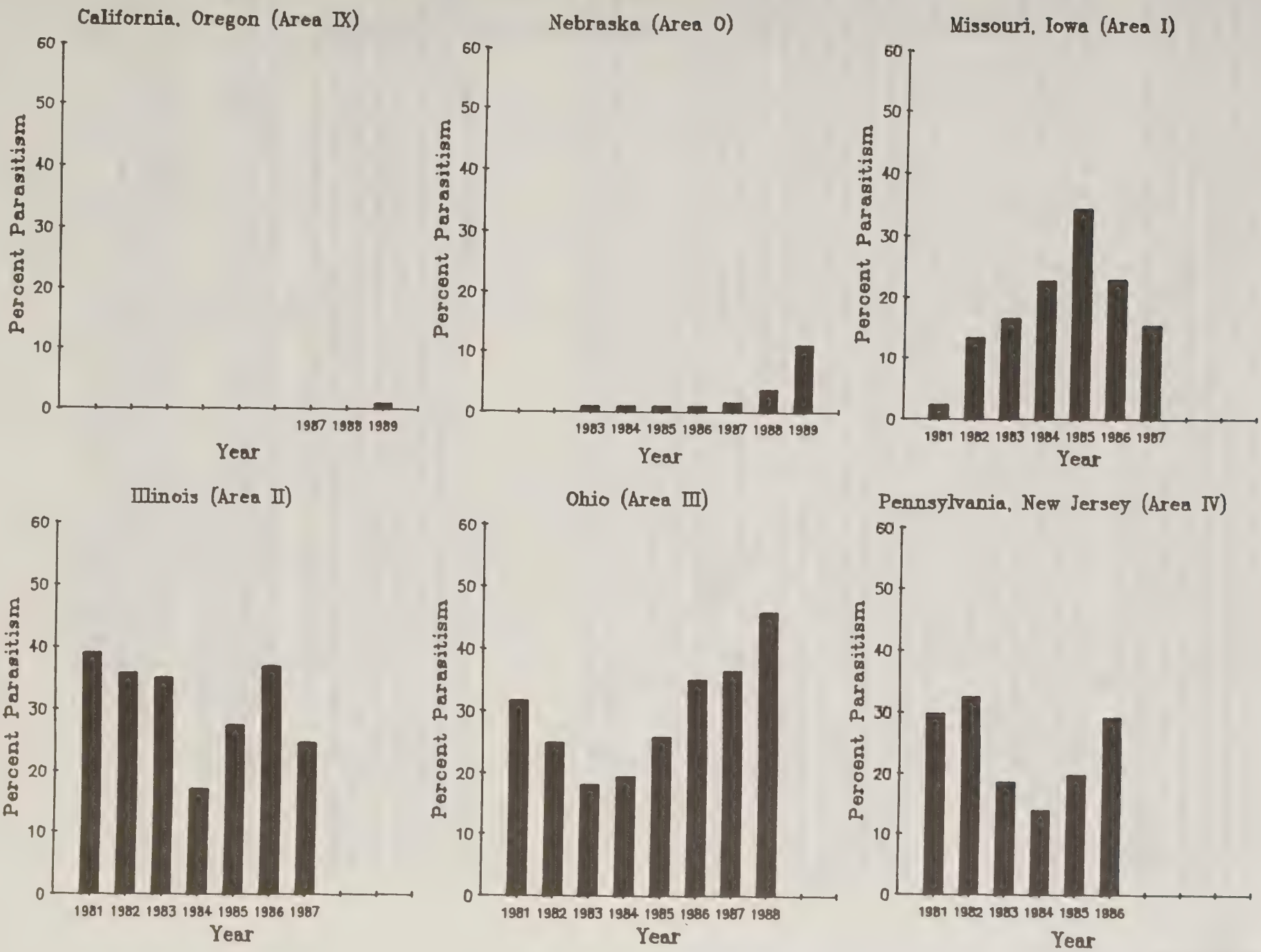


FIGURE 8.

Microctonus aethiopoides' future in the western states is questionable, however, due to an incompatibility with a rickettsia inherent to the western strain alfalfa weevil. Hsiao and Hsiao (1985b) has shown that *M. aethiopoides* cannot survive in an alfalfa weevil infected with rickettsia. Furthermore, when infected western strain weevils were treated with an antibiotic, *M. aethiopoides* survival was increased (Leu et al, 1989). Our establishment survey reflects this phenomenon. Out of 41 counties in western strain states where *M. aethiopoides* were released, only 12.2% showed establishment, compared with a 42.9% recovery rate in states dominated by eastern strain weevils (Table 1).

Several aspects of *M. aethiopoides* biology have been cited as factors leading to its important role in the alfalfa weevil parasitoid complex (Lewis, 1977; Van Driesche and Gyrisco, 1979; and Harcourt et al 1984). A key element may be its ability to sterilize female alfalfa weevils. Van Driesche and Gyrisco (1979) documented a significant reduction in oviposition preceding host mortality. Another factor is its apparent lack of hyperparasitoids (Van Driesche, 1975; Dysart and Day, 1976). Harcourt et al (1984) also noted that *M. aethiopoides* does not compete with the larval and pre-pupal disease *Erynia* sp.

Our evaluation survey indicates that *M. aethiopoides* plays a role in regulating alfalfa weevil populations by way of a delayed density dependent response to its host. This type of host/parasitoid relationship can be graphically represented by two methods. First, a delayed response can be seen when both parasitism rates, and densities of overwintering adult weevils, are plotted on the same graph by year (Figure 9 a,b,c). These data were collected from three fields that had been sampled consecutively for seven years, and were located in two counties where *M. aethiopoides* had been established prior to 1981. Clearly, parasitism rates show a similar wave pattern as the host population, but are shifted one generation to the right. This pattern of oscillating parasitism rates and host densities was modeled by Nicholson and Bailey (1935) and Hassell and Varley (1969). When these same data are plotted as parasitism against densities, and the years are connected serially, (Figure 9 d,e,f) an anti-clockwise spiral also denotes this delayed density dependent response (Hassell, 1966; Varley et al., 1973; Southwood, 1978). These data typify a reproductive numerical response of *M. aethiopoides* to its host, leading to oscillating host densities. Huffaker et al. (1971) discuss the regulating effect of a delayed density dependent parasitoid when it acts in concert with a relatively small "damping" action of a direct density dependent factor.

In Nebraska where *M. aethiopoides* is established, but still limited in terms of its impact with less than 5% parasitism, adult weevil populations are characterized by larger and more frequent fluctuations in annual densities (Figure 10).

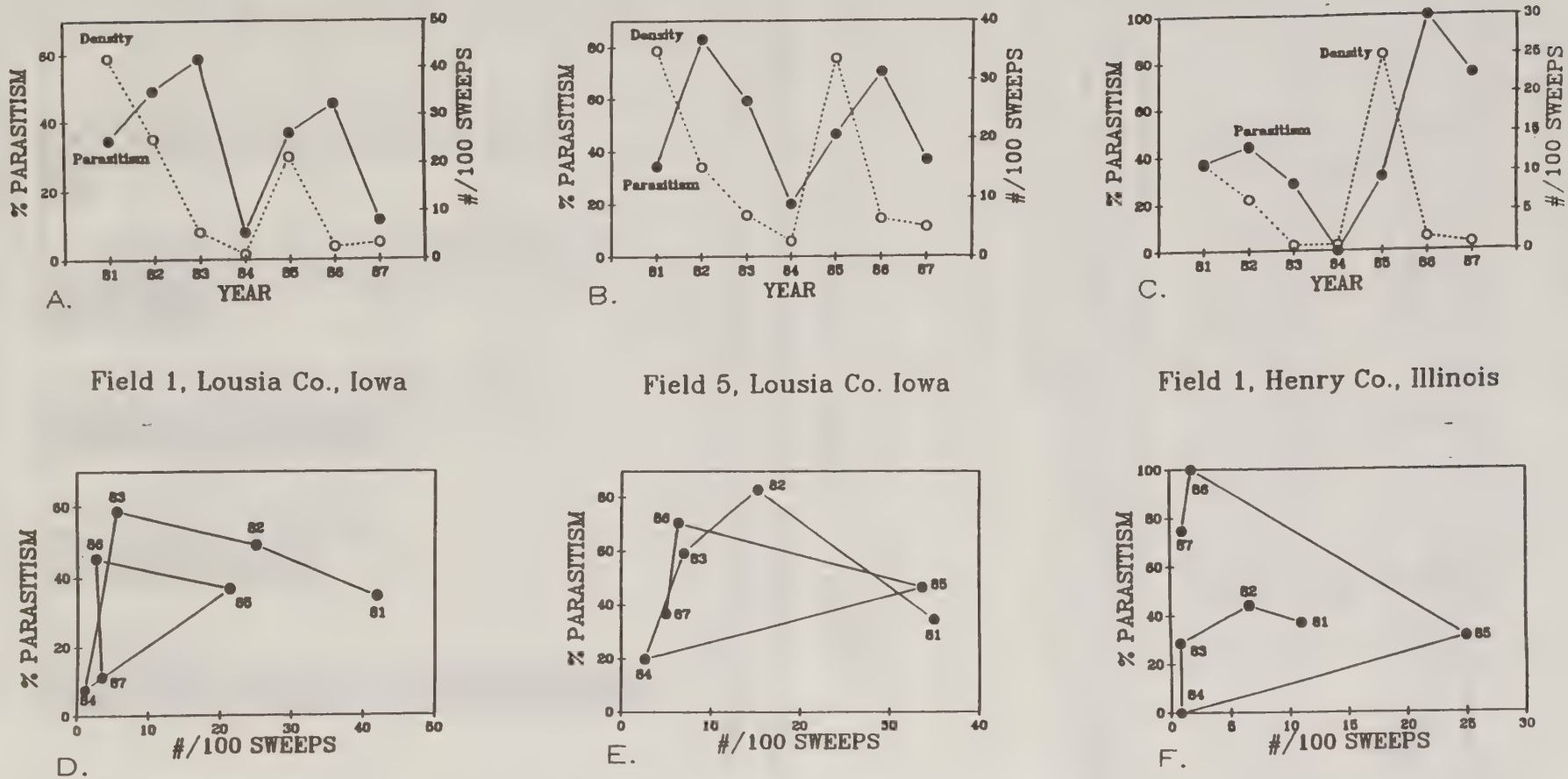
Disease

An entomogenous fungal disease of the larval and pupal stages, first detected and identified by Harcourt et al (1974) as *Entomophthora phytonomi,* was present in all areas sampled during the evaluation survey (see Yeargan 1985). The taxonomy of this pathogen is unsettled, but has most recently been identified as *Erynia* sp. by Ben-Ze'ev and Kenneth (1982), and is now commonly referred to as *Erynia phytonomi* in the literature (Johnson et al., 1984; Brandenburg, 1985). As part of the evaluation survey, we began measuring its incidence in 1984 by noting rhizoids, conidiophores, or resting spores, when dissecting samples for parasitism. This method clearly underestimates absolute disease rates, however, due to the mortality of diseased larvae in transit, but does give a satisfactory relative comparison between fields and years.

Clearly, meteorological conditions, particularly rainfall, regulate fungal disease epizootics, and therefore indirectly influence insect populations. Millstein et al. (1982, 1983 a,b) and Nordin et al. (1983) discuss microclimatic humidity influences on *E. phytonomi* conidial production, while Johnson et al. (1984) have linked rainfall to disease incidence in California.

In order to understand the influence of weather on *E. phytonomi,* and ultimately the alfalfa weevil, daily precipitation and min/max temperature data were collected from the National Weather Services' weather stations as close to each Evaluation site as possible. As expected, there was a significant positive relationship between spring precipitation and disease rates ($R^2=0.12$ $p<=.0001$ $n=135$ field years).

Overwintered Adult Alfalfa Weevils



Field 1, Lousia Co., Iowa

Field 5, Lousia Co. Iowa

Field 1, Henry Co., Illinois

Delayed Density Dependent Relationship

FIGURE 9.

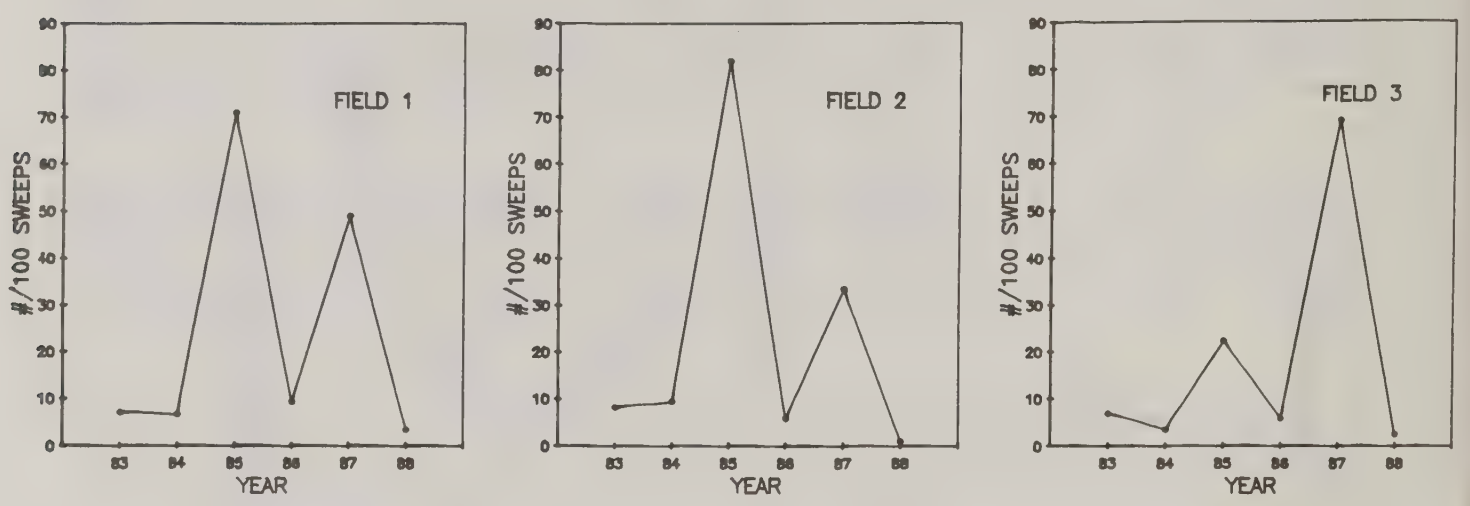


FIGURE 10.

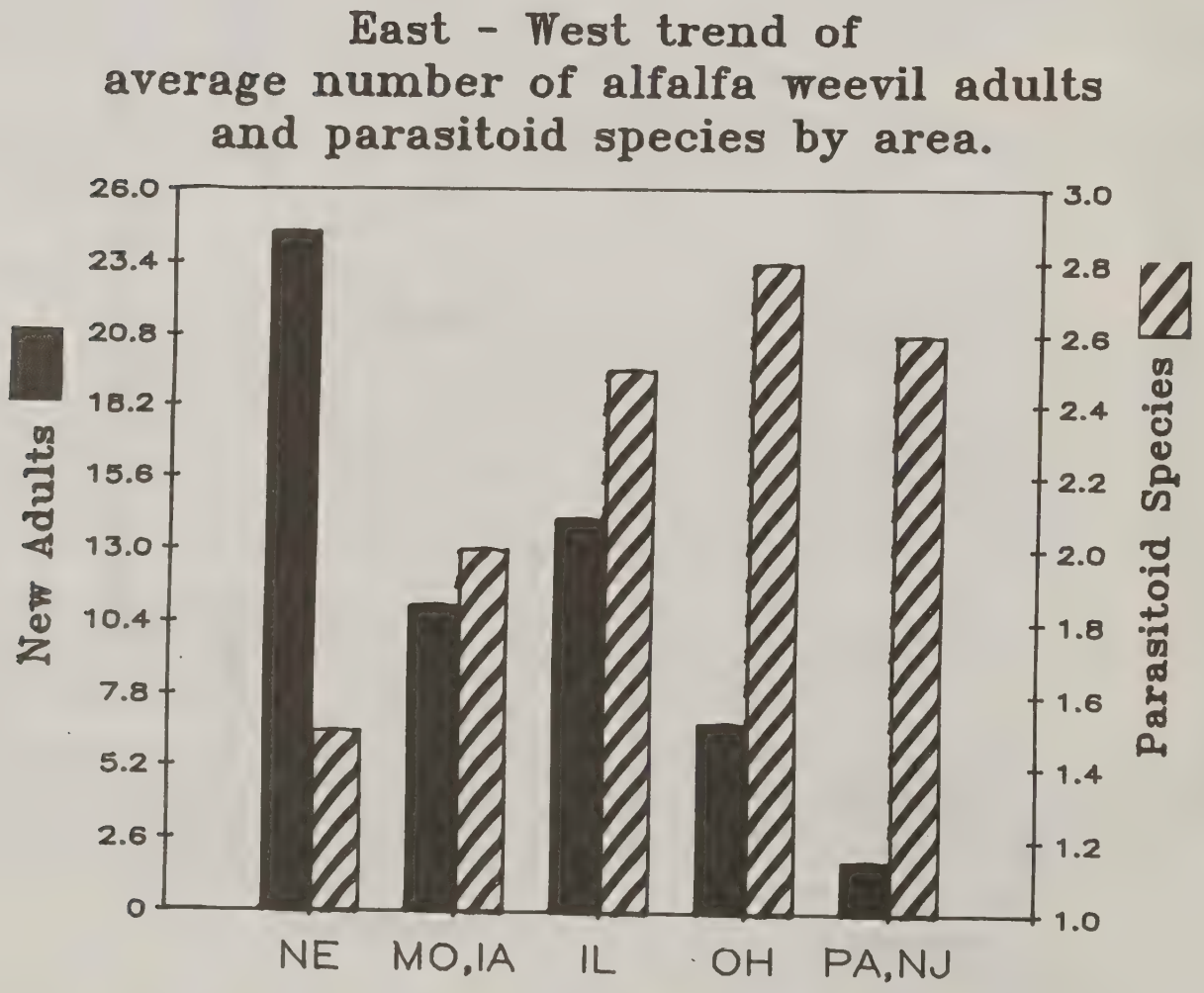


FIGURE 11.

Impact

Data from the evaluation survey sites tend to support the conclusions derived by Harcourt et al. (1977, 1984) in Ontario, Canada. They reported a significant decline in alfalfa weevil populations after the spread of *Erynia phytonomi* into the Quinte area in 1973. Extensive life table data demonstrated that this fungus was the key mortality factor regulating intra-generational weevil populations, and that it showed an overcompensating density dependent relationship with its host. The following quote from their 1984 paper describes the regulating impact of *E. phytonomi:*

> Although the disease acted in a density dependent manner, it was not entirely compensating in its action and this imposed population oscillations that were mostly coincidental in time and place. Spread of the disease was also inhibited by dry weather which delayed dissemination of the conidal spores. As a result, populations of the weevil were somewhat unstable and the likelihood of economic outbreaks tended to persist.

Erynia phytonomi was first reported from Nebraska in 1976 and Missouri in 1977 by Puttler et al (1978). They also reported severe alfalfa weevil populations of 2,030 and 1,080 per ten sweeps in two Colombia Missouri fields sampled for disease. These densities were ten times the maximal numbers encountered in any of the evaluation survey sites. Clearly, our sampling began after E. phytonomi had incurred a significant impact on alfalfa weevil populations in the Midwest.

Harcourt et al. (1984) go on to describe the regulating effect of *M. aethiopoides* and *M. colesi* in the Quinte area after these parasitoids began to parasitize significant numbers of weevils in 1977:

> Since their influx to the Quinte area, population oscillations of the weevil have been constrained, and generation survival has declined by more than 50%. As a result weevil populations have been effectively stabilized at subeconomic levels.

The increasing importance of *M. aethiopoides* can be seen in Missouri and Iowa when temporal comparisons are made between the first three and the last four years of the survey (Table 3). The number of parasitoid species increased by one during this period reflecting the establishment of *M. aethiopoides.* Mortality incurred by *M. aethiopoides* doubled after 1984 and over-wintered adult populations declined by 60%. Larval parasitism also increased but cannot be explained by the addition of *B. anurus,* as parasitism rates for this species are still insignificant in this area. There was also a significant decrease in larval densities through 1986. This difference was nullified the next year, however, when larval populations exploded, particularly in two counties. This outbreak can be explained by a drought that existed throughout the Midwest in 1987. Precipitation rates accumulated between April 1 and July 31, from the thirty Missouri/Iowa evaluation fields, were half that of the previous six year average (9.4 verses 18.2 inches). Larval populations reached their highest levels this year (787/100 sweeps) and corresponded with the lowest disease rates (2.9% compared with 11.4% for the other years measured (1984-1986)). Clearly, precipitation, or the lack of it, has a significant indirect impact on alfalfa weevil populations.

It should also be noted that a serious bias against fields with high numbers of larvae was inherent to our survey and may have influenced comparisons between larval populations. During the first three years, 27 percent (23/90) of the fields in these two states were sprayed for alfalfa weevil control, compared to only one field over the next four years (Table 3). If we assume that these treated fields never reached their maximal levels, then average larval densities for the first three years in Table 3 were clearly underestimated.

The adult weevil crop, produced from each years larval population (new adults), is a good indicator of generational survival. These density estimates, when averaged across years for each area, decline from east to west (Figure 11). Parasitoid pressure can be represented by the average number of parasitoid species recovered in each evaluation area. These data show a similar, though opposite, east west trend (Figure 11), clearly indicating that parasitoids play dominant a role in the generation to generation survival of this pest.

Temporal changes in AW populations (#/100 sweeps) and mortality rates in Missouri and Iowa (n=30 fields/year).

	1981-1983	1984-1987	
Parasitoid Species/Field	1.6 ±0.71	2.4 ** ±0.71	
AW Adult Parasitism	11.7% ±16.40	30.0% ** ±20.24	
AW Larval Parasitism	10.4% ±6.10	20.6% * ±14.18	
Overwinted AW Adults	24.1 ±26.61	8.0 ** ±16.41	
			(1984-1986)
Generational AW Larvae	385.7 ±285.65	405.5 NS ±345.24	249.9 * ±178.61
New AW Adults	9.2 ±26.15	12.2 NS ±48.16	3.1** ±5.81
Fields Sprayed for AW	25.5% 23/90	0.8% 1/120	

Two-Tailed *t* tests
NS, not significant, P>0.05;
*, significant P<0.05, P>0.01;
* *, significant P<0.001

TABLE 3.

Economic Evaluation

Information collected over the eight years of our survey showed a reduction in the number of fields sprayed for the control of the alfalfa weevil, particularly in Missouri, Iowa and Illinois (Figure 12). Cooperating farmers were also interviewed annually for information regarding the economic inputs required for alfalfa production on their farms, as well as the yields from their surveyed fields. These data were compiled by the Economic Resource Service and analyzed through a cooperative agreement with the University of Massachusetts, at Amherst. White (1989) used an econometric simulation model called AGSIM (Taylor, unpublished) to project long term benefits and costs to society, measured as economic surpluses. Joe Moffit, Geoffery Allen and Joan White then summarized these results in a report to APHIS entitled, "Economic Analysis of Alfalfa Weevil Biological Control". The conclusions of this study indicate that biological control of the alfalfa weevil has a net present value of more than $2 billion, and a benefit cost ratio of 87:1.

As noted above, alfalfa weevil larval populations rarely reached economic injury levels (22 larvae/sweep, Koehler and Rosenthal, 1975; 30 larvae/sweep, unpublished Day) during our study (Figure 13). In fact, only three percent (33/1075) of the yearly field samples were above the economic threshold. The reported reduction in insecticide use was, to a large part, due to an increase in awareness of the benefits of biological control as part of an integrated pest management approach.

Conclusions

Over 16 million parasitoids or parasitized hosts (six species) have been released in 38 states since 1980 resulting in over 700 new county records. The number of established parasitoid species per state reflects these recoveries. In Illinois, Missouri, Iowa and Nebraska for example, this number has doubled since 1981 (Figure 14). Together these data clearly indicate the success of the APHIS Redistribution Program.

The cause and effect relationships between parasitism and alfalfa weevil populations are more difficult to ascertain, however. Clearly, populations of the alfalfa weevil have been maintained, largely at sub-economic levels in the evaluation survey areas since 1981. A fungal disease, established in the early to late 1970's may have been responsible, at least in part, for this regulation. The abiotic, density independent, influence of weather on disease epizotics, and ultimately on alfalfa weevil populations, still allowed for outbreak populations. Parasitoids also cause significant annual mortality and play an important part in stabilizing the levels of this pest below those that are economic. Both *Bathyplectes* species in combination kill on average 25% of the larval generation when both are established, as in Ohio (Table 2). *Microctonus aethiopoides* also kills 25-30% of old (overwintered) adults weevils (Figure 18) in areas where it has been present for some years. Thus, up to 50-55% of each weevil generation is eliminated by parasitoids. In addition, parasitoids not measured in this study may also account for an additional 15% mortality.

Evidence that biological control is working is provided by several independent indicators: the subeconomic numbers of weevil larvae observed in the great majority of fields (Figure 13), the low and declining levels of insecticide use (Figure 12), the increasing number of parasite species with time (Figure 14), and the correlation between the rising number of parasite species and the decreasing incidence of weevil adults (Figure 11).

Percentage of fields (N=30) sprayed for AW control by area and year.

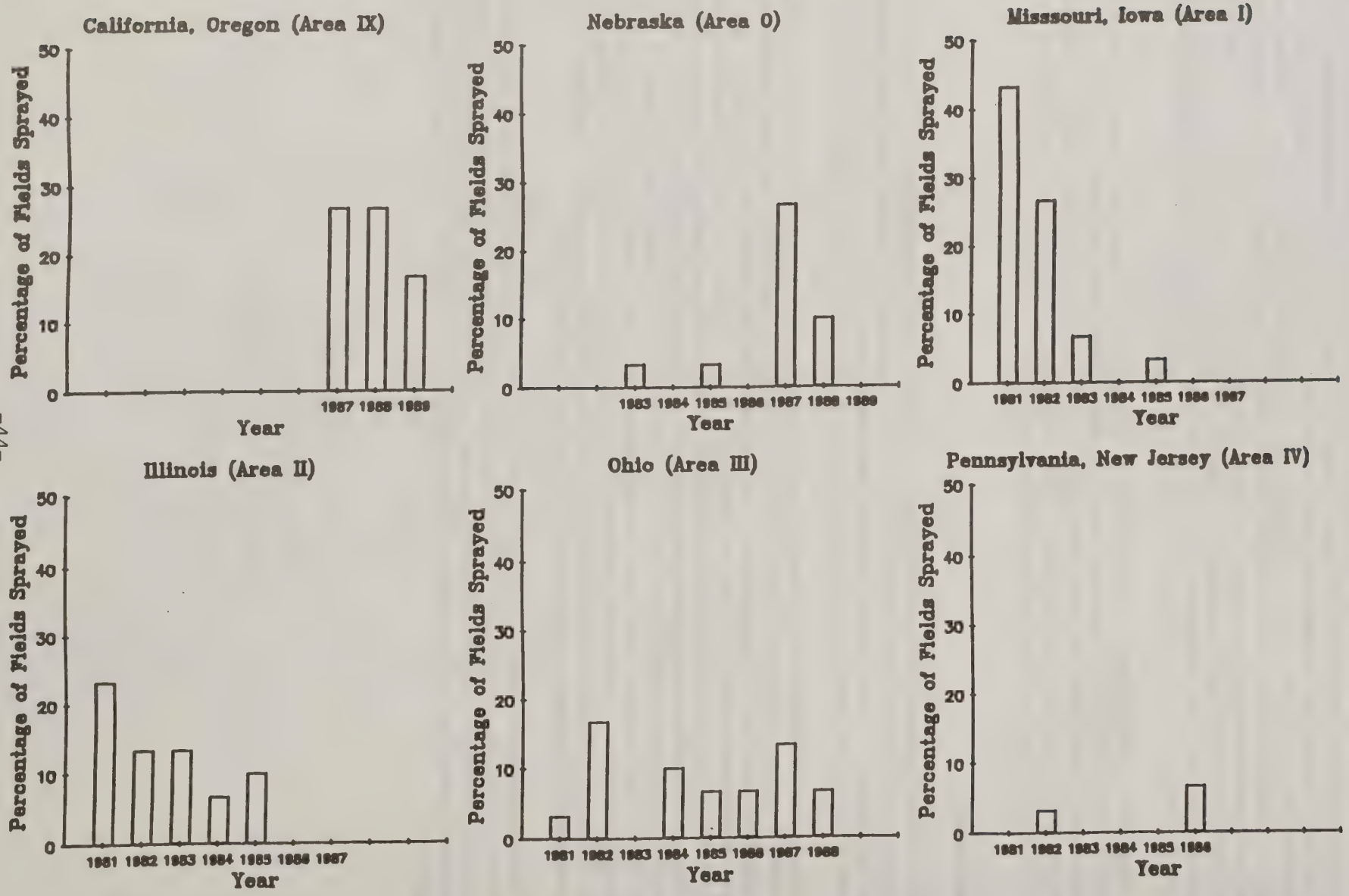


FIGURE 12.

Mean (SE) peak AW densities by area and year.

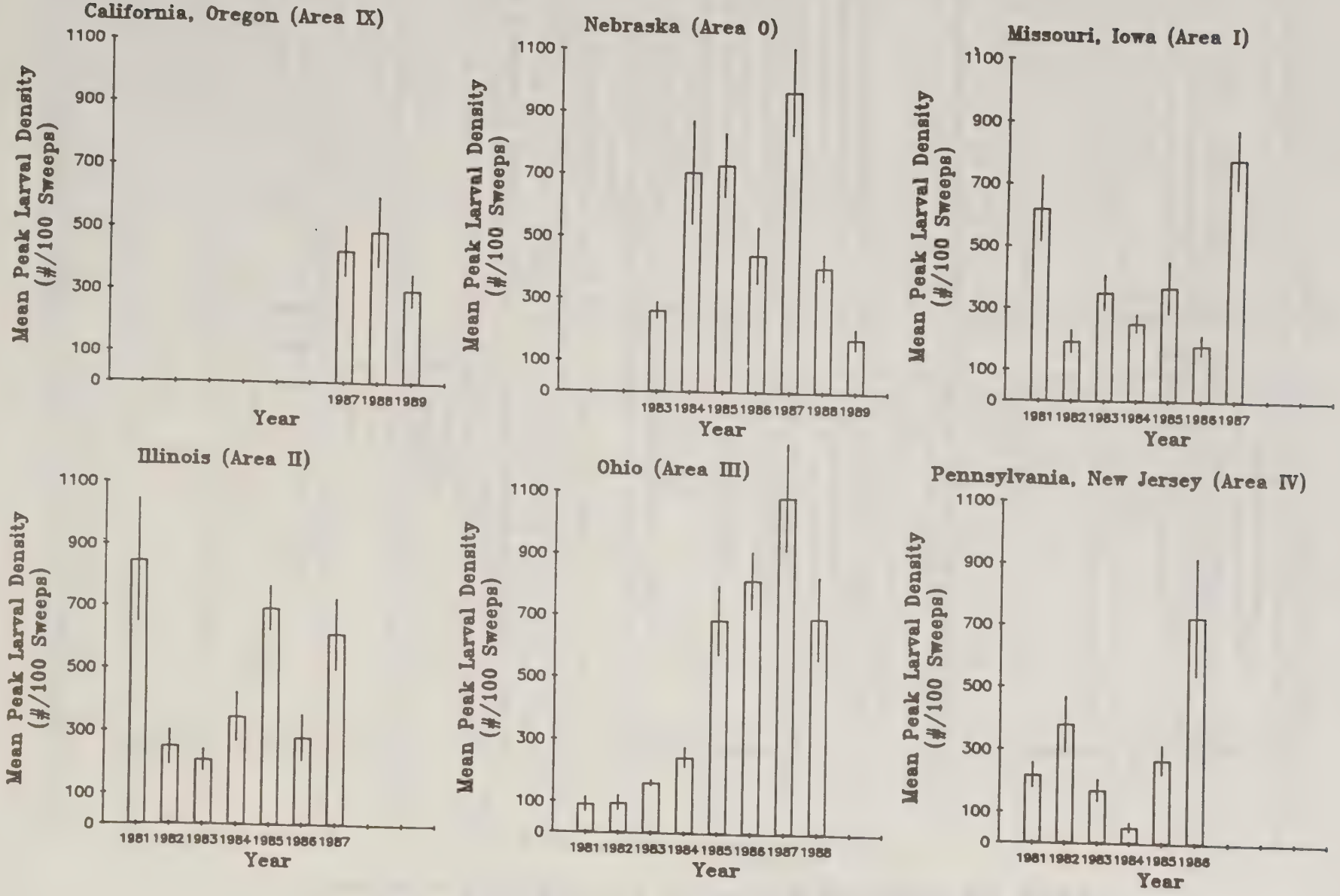


FIGURE 13.

Average number of parasite species by area and year (N=6 sites).

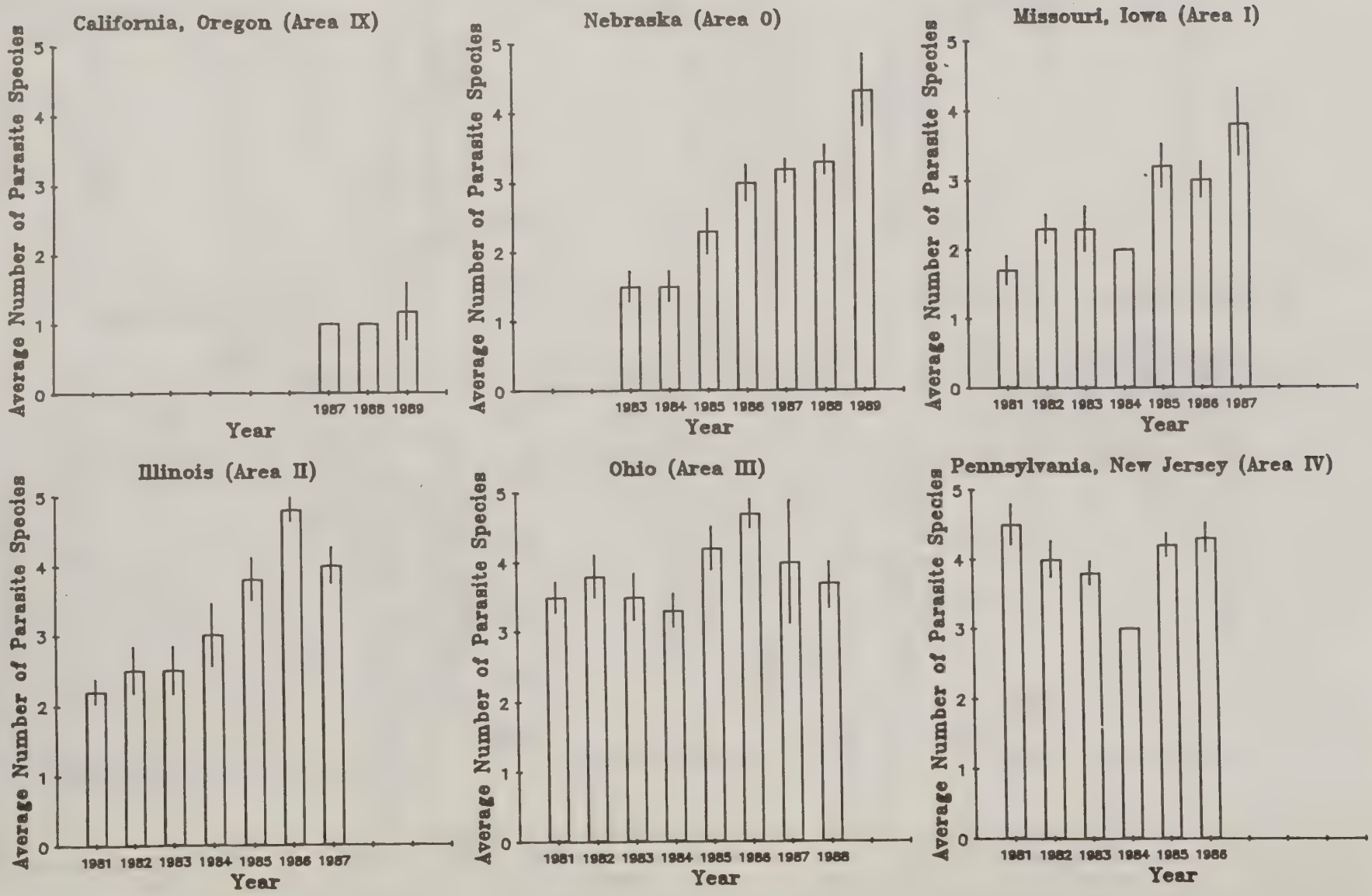


FIGURE 14.

References

Ben-Ze'ev, I. & R. G. Kenneth. 1982. Features-criteria of taxonomic value in the Entomophthorales. I. A revision of the Barkoan classification. II. A revision of the genus Erynia Nowakowski 1881 (= Zoophthora Batko 1964). Mycotaxon. 14: 393-455, 456-475.

Berberet, R. C., L. J. Willson & M. Odejar. 1987. Probabilities for encapsulation of eggs of Bathyplectes curculionis (Hymenoptera: Ichneumonidae) by larvae of Hypera postica (Coleoptera: Curculionidae) and resulting reduction in effective parasitism. Ann. Entomol. Soc. Am. 80: 483-485.

Brandenburg, R. L. 1985. Impact of Erynia phytonomi (Zygomycetes: Entomophthorales), a fungal pathogen, on alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae) populations in Missouri. J. Econ. Entomol. 78: 460-462.

Chamberlin, T. R. 1926. The introduction and establishment of the alfalfa weevil parasite, Bathyplectes curculionis (Thoms.), in the United States. J. Econ. Entomol. 19: 302-310.

Day, W. H. 1983. Incidence of nonfunctional ovaries in Bathyplectes anurus and B. curculionis (Hymenoptera: Ichneumonidae), parasites of the alfalfa weevil (Coleoptera: Curculionidae) in the northeastern United States. Environ. Entomol. 12: 1125-1128.

Day, W. H., L. W. Coles, J. A. Stewart & R. W. Fuester. 1971. Distribution of Microctonis aethiops and M. colesi, parasites of the alfalfa weevil, in the eastern United states. J. Econ. Entomol. 64: 190-193.

Dowell, R. V. & D. J. Horn. 1977. Adaptive strategies of larval parasitoids of the alfalfa weevil (Coleoptera: Curculionidae. Can. Ent. 109: 641-648.

Dysart, R. J. & W. H. Day. 1976. Release and recovery of introduced parasites of the alfalfa weevil in eastern North America. USDA Prod. Res. Rpt. 167.

Eklund, L. R. & R. G. Simpson. 1977. Correlation of activities of the alfalfa weevil and Bathyplectes curculionis with alfalfa height and degree-day accumulation in Colorado. Environ. Entomol. 6: 69-71.

Hagen, K. S., G. A. Viktorov, K. Yasumatsu & M. F. Schuster. 1976. Range, forage and grain crops, pp. 425-442. in Huffaker & Messenger [eds.], Theory and Practice of Biological Control. Academic Press, New York.

Harcourt, D. G., J. C. Guppy & M. R. Binns. 1977. The analysis of intrageneration change in eastern Ontario populations of the alfalfa weevil, Hypera postica (Coleoptera: Curculionidae). Can. Ent. 109: 1521-1534.

Harcourt, D. G., J. C. Guppy and M. R. Binns. 1984. Analysis of numerical change in subeconomic populations of the alfalfa weevil, Hypera postica (Coleoptera: Curculionidae), in eastern Ontario. Environ. Entomol. 13: 1627-1633.

Harcourt, D. G., J. C. Guppy, D. M. MacLeod & D. Tyrrell. 1974. The fungus Entomophthora phytonomi pathogenic to the alfalfa weevil, Hypera postica. Can. Ent. 106: 1295- 1300.

Hassell, M. P. 1966. Evaluation of parasite or predator responses. J. Anim. Ecol. 35: 65-75.

Hassell, M. P. & G. C. Varley. 1969. New inductive population model for insect parasites and its bearing on biological control. Nat. 223: 1133-1137.

Hsiao, C. & T. H. Hsiao. 1985. Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil, Hypera Postica. J. Invert. Path. 45: 244-246.

Hsiao, C. & T. H. Hsiao. 1984. Cytogenetic studies of alfalfa weevil (Hypera postica) strains (Coleoptera: Curculionidae). Can. J. Genet. Cytol. 26: 348-353.

Hsiao, T. H. & C. Hsiao. 1985. Hybridization and cytoplasmic incompatibility among alfalfa weevil strains. Entomol. exp. appl. 37: 155-159.

Hsiao, T. H. & J. M. Stutz. 1985. Discrimination of alfalfa weevil strains by allozyme analysis. Entomol. exp. appl. 37: 113-121.

Johnson, J. A., I. M. Hall & K. Y. Arakawa. 1984. Epizootiology of Erynia phytonomi (Zygomycetes: Entomophthorales) and Beauveria bassiana (Deuteromycetes: Moniliales) parasitizing the Egyptian alfalfa weevil (Coleoptera: Curculionidae) in southern California. Environ. Entomol. 13: 95-99.

Koehler, C. S. & S. S. Rosenthal. 1975. Economic injury levels of the Egyptian alfalfa weevil or the alfalfa weevil. J. Econ. Entomol. 68: 71-75.

Latheef, M. A., J. C. Parr & B. C. Pass. 1979. Factors affecting survival of Kentucky populations of the alfalfa weevil, Hypera postica (Coleoptera: Curculionidae). Environ. Entomol. 8: 1032-1036.

Latheef, M. A., K. V. Yeargan & B. C. Pass. 1977. Effect of density on host-parasite interactions between Hypera postica (Coleoptera: Curculionidae) and Bathyplectes anurus (Hymenoptera: Ichneumonidae). Can Ent. 109: 1057-1062.

Leu, S. C., J. K. Li & T. H. Hsiao. 1989. Characterization of Wolbachia postica, the cause of reproductive incompatibility among alfalfa weevil strains. J. Invert. Path. 54, 248-259.

Lewis, D. R. 1977. Life table analysis of alfalfa weevil population dynamics in Ohio from 1973 through 1976. UMI Dis. Inf. Ser., Michigan, 1988.

Loan, C. & F. G. Holdaway. 1961. Microctonus aethiops (Nees) auett. and Perilitus rutilus (Nees) (Hymenoptera: Braconidae), European parasites of Sitona weevils (Coleoptera: Curculionidae). Can. Ent. XCIII: 1057-1079.

Millstein, J. A., G. C. Brown & G. L. Nordin. 1982. Microclimatic humidity influence on conidial discharge in Erynia sp. (Entomophthorales: Entomophthoraceae), an entomopathogenic fungus of the alfalfa weevil (Coleoptera: Curculionidae). Environ. Entomol. 11: 1166-1169.

Millstein, J. A., G. C. Brown & G. L. Nordin. 1983. Microclimatic moisture and conidial production in Erynia sp. (Entomophthorales: Entomophthoracaea): in vivo production rate and duration under constant and fluctuating moisture regimes. Environ. Entomol. 12: 1339-1343.

Millstein, J. A., G. C. Brown & G. L. Nordin. 1983. Microclimatic moisture and conidial production in Erynia sp. (Entmophthorales: Entomophthoraceae): in vivo moisture balance and conidiation phenology. Environ. Entomol. 12: 1339-1343.

Nicholson, A. J. & V. A. Bailey. 1935. The balance of animal populations. Part I., Proc. zoo. Soc. Lond. 1935: 551-598.

Nordin, G. L., G. C. Brown & J. A. Millstein. 1983. Epizootic phenology of Erynia disease of the alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae), in central Kentucky. Environ. Entomol. 12: 1350-1355.

Pike, K. S. & C. C. Burkhardt. 1974. Hyperparasites of Bathyplectes curculionis in Wyoming. Environ. Entomol. 3: 953-956.

Puttler, B. 1967. Interrelationship of Hypera postica (Coleoptera: Curculionidae) and Bathyplectes curculionis (Hymenoptera: Ichneumonidae) in the eastern United States with particular reference to encapsulation of the parasite eggs by the weevil larvae. Ann. Entomol. Soc. Am. 60: 1031-1038.

Puttler, B. 1974. Hypera postica and Bathyplectes curculionis: encapsulation of parasite eggs by host larvae in Missouri and Arkansas. Environ. Entomol. 3: 881-882.

Puttler, B., D. L. Hostetter, S. H. Long & R. E. Pinnell. 1978. Entomophthora phytonomi, a fungal pathogen of the alfalfa weevil in the mid-great plains. Environ. Entomol. 7: 670-671.

Rethwisch, M. D. & G. R. Manglitz. 1986. Parasitoids of Bathyplectes curculionis (Hymenoptera: Ichneumonidae) in southeastern Nebraska. J. Kan. Ent. Soc. 59: 648-652.

Southwood, T. R. E. 1978. Ecological Methods. 2nd. ed., pp. 524. Chapman & Hall, London.

Southwood, T. R. E. & W. F. Jepson. 1962. Studies on the populations of Oscinella frit. L. (Diptera: Chloropidae) in the oat crop. J. Animal Ecology. 31: 481-495.

Van Den Bosch, R. & E. J. Dietrick. 1959. The interrelationships of Hypera brunneipennis (Coleoptera: Curculionidae) and Bathyplectes curculionis (Hymenoptera: Ichneumonidae) in southern California. Ann. Ent. Soc. Am. 52: 609-616.

Van Driesche, R. G. 1975. An evaluation of Microtonus aethiops (Nees) (Hymenoptera: Braconidae) as a parasitoid of the adult alfalfa weevil, Hypera postica. UMI Dis. Inf. Ser., Michigan, 1988.

Van Driesche, R. G. & G. G. Gyrisco. 1979. Field studies of Microctonus aethiopoides, a parasite of the adult alfalfa weevil, Hypera postica, in New York. Environ. Entomol. 8: 238-244.

Varley, G. C., G. R. Gradwell & M. P. Hassell. 1973. Insect Population Ecology.pp. 212. Blackwell Scientific Publications, Oxford.

White, J. M. 1989. The effects of biological control of the alfalfa weevil; an economic welfare analysis. U. Mass. Dept. Agri. Res. Econ.

Yeargan, K. V. 1985. Alfalfa: status and current limits to biological control in the eastern U. S.,pp. 521-536. in Hoy and Herzog [eds.], Biological Control in Agricultural IPM Systems. Academic Press, New York.

Yeargan, K. V. & B. C. Pass. 1978. Description and incidence of non-functional ovaries in Bathyplectes curculionis. J. Kansas Entomol. Soc. 51: 213-217.

Project Number: BT 87.1.1
Project Title: Browntail Moth Rearing Techniques
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leader: A. Pellegrini-Toole, V. Mastro, B. Ronberg

This year only seven groups of webs were infested and reared. Adult females were used for gland tip extracts; adults males were discarded. Due to a successful trapping season, indicating that the pheromone has been correctly synthesized, we will no longer need to rear browntail moths in the laboratory.

Trapping results will be detailed in a separate report.

Project Number: BT 89.1.1
Project Title: Sex pheromone of the browntail moth *Euproctis chrysorrhoea* (L.): Field evaluation and diel periodicity of production by females.
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leaders: V. C. Mastro and A. Pellegrini-Toole
Cooperators: B. Leonhardt and M. Schwarz

Introduction

The browntail moth *Euproctis chrysorrhoea* (L.) (Lepidoptera:Lymantriidae) is native to Eurasia. Within its native range it has a history of periodic outbreaks which result in defoliation of forest, shade and orchard trees. The host range of the browntail is broad (Schaefer 1974) with preferred host in the families Rosaceae and Fagaceae. In addition to the defoliation damage caused by larval feeding activity, the setae of larvae contain toxins which, when contacting skin, result in a severe dermatitis. This pest was accidentally introduced into North America sometime prior to 1897. In that year it was first discovered when residents of Somerville and Cambridge, Massachusetts noticed damage on their pear trees (Fernald and Kirkland 1903). Its range expanded rapidly and by 1914 populations of browntail were known to occur in seven northeastern states and two Canadian provinces (Burgess, 1915). It is interesting to note that at this time its range surpassed that of another imported lymantriad, the gypsy moth *Lymantria dispar* (L.), which had been introduced approximately 30 years prior to the browntail moth.

Although more than eighty years have passed since the initial browntail moth population outbreak, there has not been a reoccurrence; in fact since 1918 its apparent range has diminished until presently, the only known populations occur on Cape Cod, Massachusetts and on several islands in the Casco Bay area of Maine. Although several explanations have been offered, there is no clear evidence for the cause of this population contraction (Schaefer 1974, 1986). In recent years, however, densities, and, to a limited extent, the apparent range of the remnant populations, have increased (P. Snowden 1986; D. Strubel, personal communication). Browntail moth populations within the Cape Cod National Seashore have expanded their range from secondary coastal dunes to inland areas, and from primarily dune vegetation, beach plum (*Prunus maritima*) and black cherry (*Prunus serotina*) to upland hosts (Leonard 1986).

Although in Europe light traps (Szontagh 1974) have been used for assessing populations, their widespread use was never adopted in the United States. Visual detection surveys in the New England states were carried out during the fall and winter, when the silken communal webs are most apparent on the terminal branches of host plants. While visual surveys were effective when populations were relatively dense, they demanded large investments of manpower. The United States Department Agriculture Animal and Plant Health Inspection Service discontinued survey in 1971. The existence of a female attractant has been known for a long time (Komarek and Pfeffer 1939). Female baited traps have been used for survey and studies of the moth's behavior (Jacobson 1972, Schaefer 1974). Identification of the browntail moth's pheromone would permit development of an efficient survey tool for monitoring the density and distribution of populations.

Preliminary investigations and observations in 1985 and 1986 resulted in confirmation that females produce a pheromone. Analysis of extracts of the terminal two female abdominal segments resulted in identification of a single major attractant compound (Leonhardt et al, 1990). A gas chromatograph with a retention time of 19.97 using a florisil column was bioassayed in a flight tunnel and by using electroantennograms. This led to the identification of the compound as (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate. This material was synthesized by one of the authors (Schwarz 1990) and first field tested in 1987. The studies reported below were carried out between 1987 and 1989.

Field Evaluations

Field comparisons of live females, abdominal tip extracts, and the synthetic compound were carried out on the Cape Cod National Seashore. Throughout the tests, wing type traps (Pherocon P1C) or similar traps were used for all treatments. Traps were modified to accommodate baiting with live females by the addition

of a small wire cage (4 cm. ht. X 7 cm. dia. X 32 mesh between the two trap halves). For placement, traps were suspended from vertical steel rods (8mm. dia. X 1.7m.) driven into the ground (ca. 0.3m). Generally the test design was a randomized complete block and also generally traps were checked and re-randomized daily.

Filter paper disks (22mm dia.) or sections of cotton dental rolls (6mm X 13mm dia.) were used as dispensers for both synthetic compounds and extracts of female abdominal tips in 1987 and 1988. In 1989 modified rubber septa (West Co.) were used as dispensers. Septa were modified by removing the upper half of the cup end. Trapping data were analyzed using analysis of variance, and means were separated using Duncan's Multiple Range technique.

Females

Females used for baiting traps and for pheromone extraction were collected as pupae from naturally infested areas within the Cape Cod National Seashore. Pupae were sexed and held in separate containers for eclosion at 25°C, 40%RH, 16:8 L:D. Eclosion containers were checked daily and newly eclosed adults were removed and held separately.

For gland extraction, females were held until the day following eclosion (1 day old). The terminal abdominal segments were everted by lightly squeezing the abdomen and the terminal two segments were excised for extraction. Abdominal tips were immersed in re-distilled hexane for 3-4 minutes after which the supernatant solution was pipetted into conical microproduct vials which were sealed with Teflon-lined screw caps. Female whole body extracts were also made by immersing the female in hexane 3-4 minutes. As for extracts of abdominal tips, the supernatant was pipetted into vials and kept frozen until use. Samples were kept frozen (-17°C) until chemical analysis was carried out. Concentration of (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate in tip extracts was determined on a gas chromatograph. A series of female tip extractions were done at intervals throughout the day to investigate the diel periodicity of pheromone level in the gland.

Results and Discussion

Early field trials 1987-1988 with traps baited with female gland extracts or the synthetic compound (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate resulted in poor male captures. Traps baited with the synthetic compound or extracts usually captured some males during the first night that the traps were deployed; however, in subsequent nights, male capture fell dramatically compared to capture in female baited traps (Table 1). In these early trials, filter paper disks were used as dispensers for all materials. We speculated that the active compound was being released too rapidly from dispensers. Laboratory analysis of aged wicks confirmed this theory.

Table 1. Captures of male *E. chrysorrhoea* in traps baited with live females (3/trap) and (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate - 1987

TREATMENT	Mean daily male capture for 3 replicates	
	DAY 1	DAY 2
1 day old females	66.3	40.0
2 day old females	65.3	43.0
50µg synthetic [1/] (Z,Z,Z,Z) 7,13,16,19	9.3	0.3
Blank	0.0	0.25

[1/] filter paper disk (22 mm dia.) used for dispenser

In 1989, traps baited with 25 μg to 50 μg of (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate dispensed on rubber septa captured males in numbers similar to traps baited with virgin females (Tables 2 and 4). Generally increasing dispenser loading rates resulted in captures of increasing numbers of male browntail moths (Table 2, 3 and 4). The highest loading rate tested (250 μg), however, did not increase trap capture relative to the next lower loading rate (50μg) (Table 3). Emission rate studies with dispensers indicate that the compound is emitted at a relatively high rate and perhaps at the highest dispenser loading the rate is beyond the optimum level. Similarly, traps baited with increasing concentrations of the natural compound (determined by GC) caught increasing numbers of males (Table 4). Also, similar dispenser loadings of the synthetic compound and female gland extracts produced very similar trap captures.

Traps baited with the configurational isomer (E,Z,Z,Z)-7,13,16,19- docosatetraenyl isobutyrate captured significantly fewer males than traps baited with an equal loading of the Z,Z,Z,Z isomer (Tables 2 and 3). Also, when 50 μg of a 1:1 mixture of the two isomers were used to bait traps, numbers of males captured were less (not significantly different) than traps baited with 50 μg of only the Z,Z,Z,Z isomer. Addition of the alcohol precursor, to the synthetic (Z,Z,Z,Z)-7,13,16,19 docosatetraenyl isobutyrate, to dispensers did not significantly improve trap performance (Table 3). Unfortunately, only a limited amount of the alcohol was available and it was not tested alone as an attractant.

Female Pheromone Gland Content

The highest concentrations of (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate were found in gland extracts of females processed at 8:00 - 10:00 PM (Figure 1). The highest concentration found was 23 ng/female. Concentrations decreased after 10:00 PM but remained relatively high throughout scotophase. Concentrations decreased sharply when lights went on (5:00 AM) and remained low throughout the photophase period until just prior to lights out (9:00 PM). In our laboratory studies some females were observed displaying calling behavior two hours before lights out. However, most females did not initiate calling until 3-4 hours after lights out. Schaefer (1974) reports that flight activity of both sexes is initiated at dusk. Apparently, female flight activity during this period is largely by fertilized females. Males in Schaefer's studies displayed three peak activity periods: dusk, near midnight, and a pre-dawn period. However, males were captured in traps baited with females consistently only near midnight and during the pre-dawn period. Our extraction of pheromone glands indicates that just prior to the male activity peak, female pheromone concentrations are at their highest.

Although from the previously described results we believe that (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate is the major component of the female browntail moth pheromone, it is possible that other compounds are involved. Thus far, however, electroantennogram studies with GC fractions of gland extracts have only shown activity at one peak. In the genus *Euproctis* only one other species pheromone has been identified. The pheromone for *E. similis xanthocampa* was identified by Tan et al (1984) as Z-7 octadecenyl isovalerate. Priesner (1975) in his electroantennagram studies of male *E. chrysorrhoea* and *E. similis* responses to female gland preparations of both species demonstrated strong reciprocal responses. Recently, however, taxonomic placement of *similis* in the genus *Euproctis* has been questioned (Maes 1984). This revision would place *similis* in the genus *Sphrageidus*. In 1988 field trials we tested Z7-18 isovalerate as an attractant for browntail males. In these earlier field trials 22mm dia. disks of filter paper were used as dispensers for compounds. During two days of testing, traps baited with virgin females averaged 40 males captured per night while traps baited with Z-7-18 isovalerate (50 μg) captured an average of 6.5 males per night and traps baited with (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate captured a mean of 19.3 males per night. Interestingly, a relatively common compound of squalene (2,6,10,15,19,23-hexamethyl 2,6,10,14,18,22 tetracosahexaene) baited traps (50 μg) displayed some field activity capturing an average of 4.8 males per night.

Of the few species in the family Lymantriidae for which pheromones have been identified (Arn, 1986), the identified compounds are all relatively large, compared to compounds identified for other Lepidoptera species. The uniqueness of the pheromone compounds so identified for this family may not be surprising, given other unique characteristics of its member species (Schaefer, 1989).

Table 2. Capture of male *E. chrysorrhoea* in traps baited with (Z,Z,Z,Z) and (E,Z,Z,Z) isomers of 7,13,16,19 docosatetraenyl isobutyrate, virgin females and extracts of female glands in field trials on the Cape Cod National Seashore 7/16 - 7/17/89.

ATTRACTANT SOURCE	MEAN NO [1/] MALES CAPTURED	SPLIT
(Z,Z,Z,Z) 7,13,16,19 50μg	30.3	a
(Z,Z,Z,Z) 7,13,16,19 25μg	18.3	bc
(Z,Z,Z,Z) 7,13,16,19 5μg	18.3	cd
(Z,Z,Z,Z) 7,13,16,19 0.5μg	2.3	fg
(E,Z,Z,Z) 7,13,16,19 25μg	9.3	de
1:1 (Z,Z,Z,Z):(E,Z,Z,Z) 50μg	26.8	ab
female gland extracts 0.5μg	5.0	ef
virgin females	25.5	ab
Blank	1.0	g

1/ Means followed by the same letter are not significantly different at P=5% according to Duncan's NMRT after ($\sqrt{n}$ + 0.5) transformation of data.

Table 3. Captures of male *E. chrysorrhoea* in traps baited with (Z,Z,Z,Z) and (E,Z,Z,Z) isomers of docosatetraenyl isobutyrate, its alcohol precursor, and female gland extracts in field trials on the Cape Cod National Seashore 7/18- 7/27/89

ATTRACTANT SOURCE	MEAN NO [1/] MALES CAPTURED
(Z,Z,Z,Z) 7,13,16,19 250μg	5.5 ab
(Z,Z,Z,Z) 7,13,16,19 25μg	8.3 a
(Z,Z,Z,Z) 7,13,16,19 2.5μg	4.1 b
(E,Z,Z,Z) 7,13,16,19 25μg	1.5 c
(1:1) (Z,Z,Z,Z) 7,13,16,19:alcohol 50μg	6.6 ab
female gland extract 0.1μg	0.3 c
blank	0.2 c

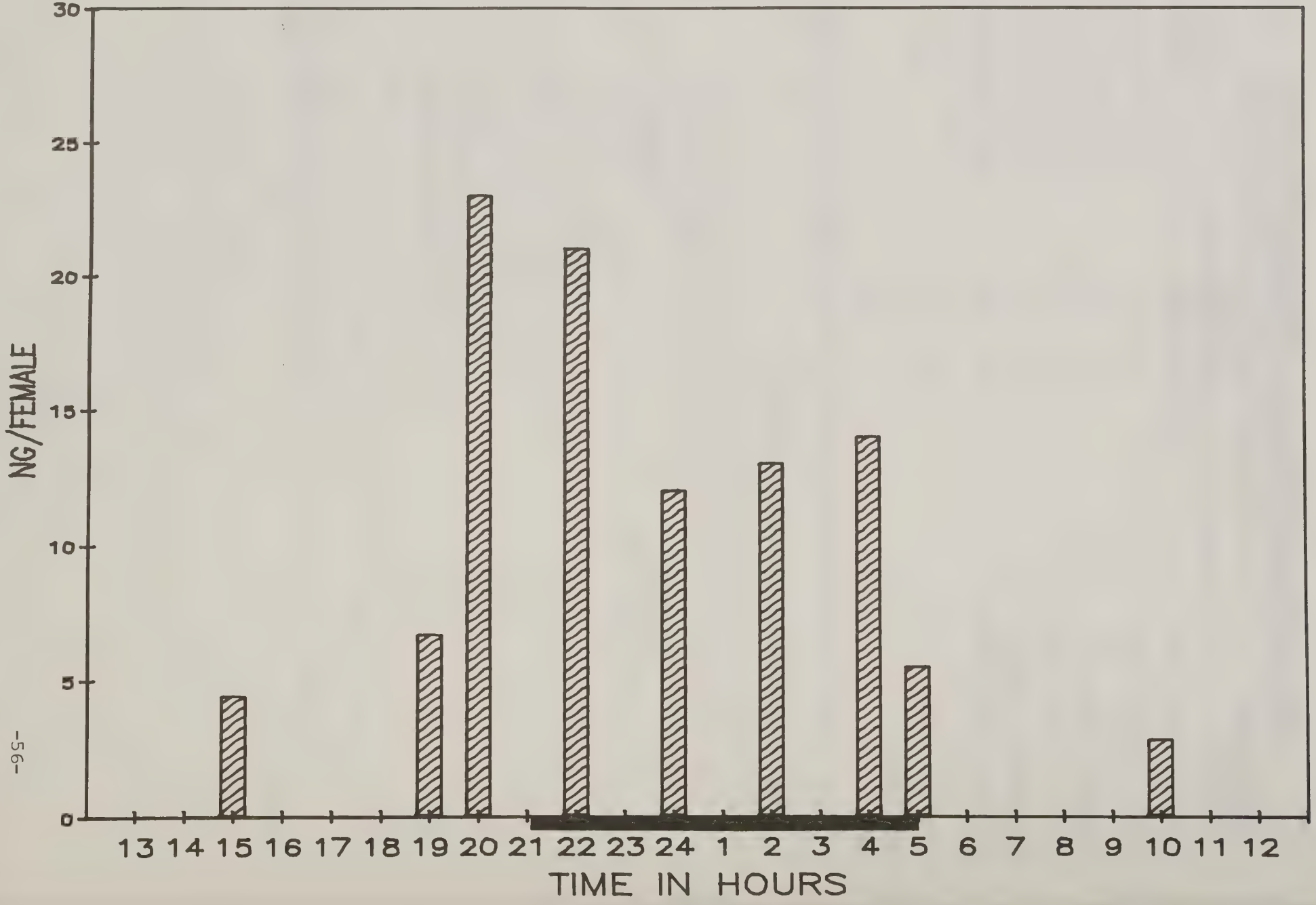
1/ Means followed by the same letter are not significantly different at P=5% according to Duncan's NMRT after ($\sqrt{n}$ + 0.5) transformation of data.

Table 4. Captures of male *E. chrysorrhoea* in traps baited with (Z,Z,Z,Z) 7,13,16,19 docosatetraenyl isobutyrate, females and female gland extracts.

ATTRACTANT SOURCES	MEAN NO [1/] MALES CAPTURED
(Z,Z,Z,Z) 7,13,16,19 25μg	18.2 a
(Z,Z,Z,Z) 7,13,16,19 5μg	12.1 b
(Z,Z,Z,Z) 7,13,16,19 1μg	3.8 c
(Z,Z,Z,Z) 7,13,16,19 0.2μg	0.8 d
(Z,Z,Z,Z) 7,13,16,19 0.04μg	0.3 d
Gland extracts 1μg	3.4 c
Whole body extracts 0.4μg	0.8 d
Gland extracts 0.1μg	0.2 d
Whole body extracts 0.04μg	0.3 d
Virgin females	14.9 ab
Blank	0.3 d

1/ Means followed by the same letter are not significantly different at P=5% according to Duncan's NMRT after ($\sqrt{n + 0.5}$) transformation of data.

Figure 1. Concentration of Z,Z,Z,Z-7,13,16,19 docosatetraenyl isobutyrate in extracts of excised female browntail abdominal tips.



Project Number: CAPS 84.1.1
Project Title: Trap design studies with the apple ermine moth *Y. malinellus*
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leaders: V. C. Mastro, E. Lagasa, Washington State Dept. of Agriculture

The apple ermine moth (AEM) *Yponomeuta malinellus* is an exotic pest of *Malus* species that has been introduced into the western portion of the state of Washington and British Columbia. Recent identification of the pheromone (McDonough, 1988) for this species has permitted delimitation surveys within Washington and detection surveys in other states. Because so little time has elapsed since the identification of the pheromone, no trap design studies have been carried out. In an attempt to find a trap design that performed better than the standard (wing-type trap) used in the 1989 survey program, a small study was carried out in Bellingham, Washington. A second objective of this study was to find a "dry" type (not a sticky catching mechanism) trap. A "dry trap" would ease identification problems with captured specimens.

Traps were placed in host trees (apple) in residential areas. Because of the host tree location, an equal between-trap spacing was not maintained. Four complete replicates of the twelve trap designs were deployed, read and randomized twelve times. All traps were baited with lures from the Otis Methods Development's 1989 production lot.

Results of this test demonstrate that the trap design which results in the largest numbers of males captured is the Multipher-3 (Table 1). This is a "dry" type trap and provides for a volume of male apple ermine moths that would not be exceeded even in trapping dense populations. Perhaps this trap's one drawback may be its high initial cost. However, the construction would permit its use over many years. If a lower-cost disposable trap were desired, the Pherocon IC or INRA type delta should be chosen.

Figures 1 through 3 illustrate trap designs which are not of commercial or standard USDA design.

Results of an apple ermine moth *Yponomeuta malinellus* trap design comparison conducted in Bellingham, Washington, 7/12 - 8/31, 1989.

Trap	Mean No. of males captured[1]/trap/reading	
Wing trap (Pherocon 1C)	9.3	b
Delta (USDA - std.)	0.6	e
Delta (USDA - ends open)	3.6	c
Pup tent (large)	3.4	cd
Pup tent (small)	2.8	cd
Large delta (INRA)	9.4	b
Milk carton (USDA - std.)	2.1	d
Multipher-1	7.9	b
Multipher-3	13.9	a
Cono-cup (USDA exp.)	3.3	cd
Jug trap (USDA exp.)	0.3	e
Hercon exp.	0.5	e

1/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's New Multiple Range Test. Four complete replicates were read and randomized twelve times.

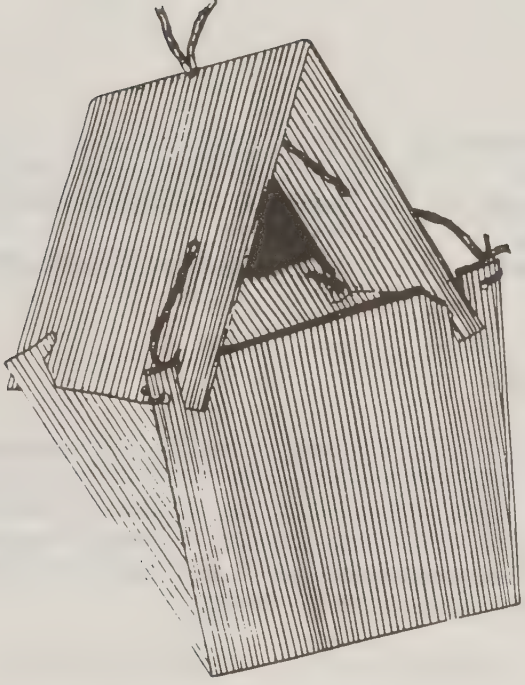


Fig. 1 - Pup tent; Briese experimental trap.

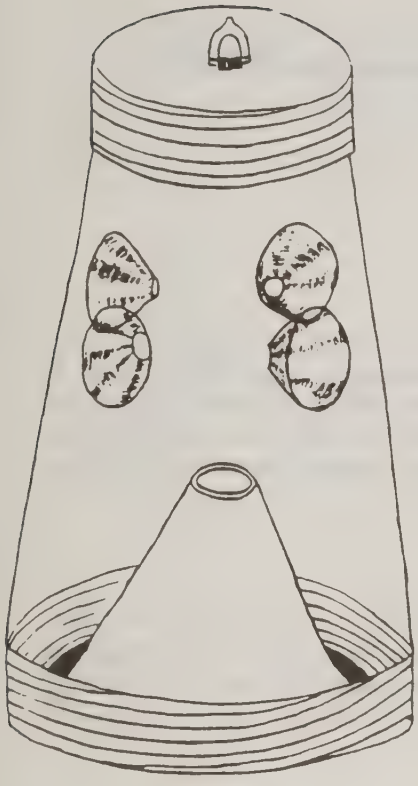


Fig. 2 - Hercon exp. - Hercon Corp. experimental trap.

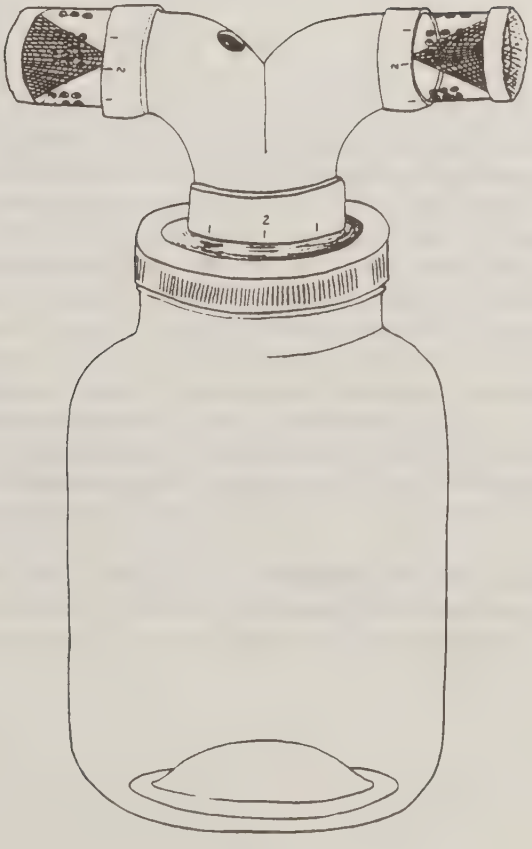


Fig. 3 - Jug trap - USDA experimental trap.

Project Number: CPB 1.1.1
Project Title: Rearing *Edovum puttleri*, an egg parasitoid of *Leptinotarsa decemlineata*
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leader: Philip C. Kingsley, Robert James, Cathy Paris

Introduction

As part of an overall objective to develop efficient rearing methods for *Edovum puttleri* (Eulophidae) (see CPB 1.1.1 1986, 1987, 1988 interim reports) it is important to quantify Parasitoid/Host (P/H) ratios in oviposition cages. These optimal parasitoid host ratios depend primarily on two factors: parasitoid age and the amount of interference between females. This is clearly because both have a direct influence on fecundity. Lashomb et al. (1987) described the mathematical relationship between age and the daily fecundity of *E. puttleri* with the formula:

$$EGGS/DAY = -4.19 + 2.42\ AGE - 0.09\ AGE^2.$$

Varley et al. (1973) stated that "Searching parasites may change their behavior if other individuals of the same species are nearby or after they have detected a parasitized host. Such behavior tends to result in a decreasing searching efficiency as parasite density increases.". This concept was originally described as a species' "area of discovery" by Nicholson and Bailey (1935) then later by Hassell and Varley (1969) and can be described using the formula:

$$\log a = \log Q - m \log P$$

where Q is the "quest constant" or the area of discovery when P = 1, m is "the mutual interference constant" or the slope of this equation, and P is the parasite density.

The objective of this study was to study the relationship of age and parasitoid density to parasitoid/host ratios.

Methods

These tests were replicated twice, the first during November 1988, and the second in January 1989. Small oviposition cages (~130cm^3) were designed from specimen containers fitted with moist dental wicks. In both tests host eggs were supplied daily. In test I all eggs were removed from leaves using hexane (see 1987 CPB Interim report), glued to filter paper, then attached to the cage tops using a small plastic petri dish and paraffin. As part of the second test, half of the host eggs were left on the leaves to allow for a comparison with extracted eggs. A small smear of honey was also provided on the cage top as a food source. In order to achieve various P/H ratios each cage (22 cages in test I and 23 in test II) was set up with different numbers of wasps, from 6 to 35 (Mean = 11.7 ±0.26). These wasps had emerged within one day. Each day, as host eggs were replaced, the number of wasps were counted and dead wasps were removed and sexed. This allowed us to determine at the conclusion of the experiment (26 days in I and 19 days in II), how many females were in each cage each day. In addition, the number of eggs supplied daily varied from 8 to 156 and averaged 40.1 ±1.02 per cage. Using this experimental design we achieved P/H ratios ranging from 0.03 to 2.1 (Mean = 0.37 ±0.01) for female *E. puttleri* between the ages of 1 and 26 days.

All progeny from the first experiment were reared to adults although parasitism rates were based on the number of eggs reaching the later stages of parasitism. Host eggs from half of the cages in the second test were frozen for dissection, allowing for the identification of the actual number of eggs laid and superparasitism.

Figure 1. Parasitism, as measured by k-values, in relation to parasite/host ratios (Mean;SE).

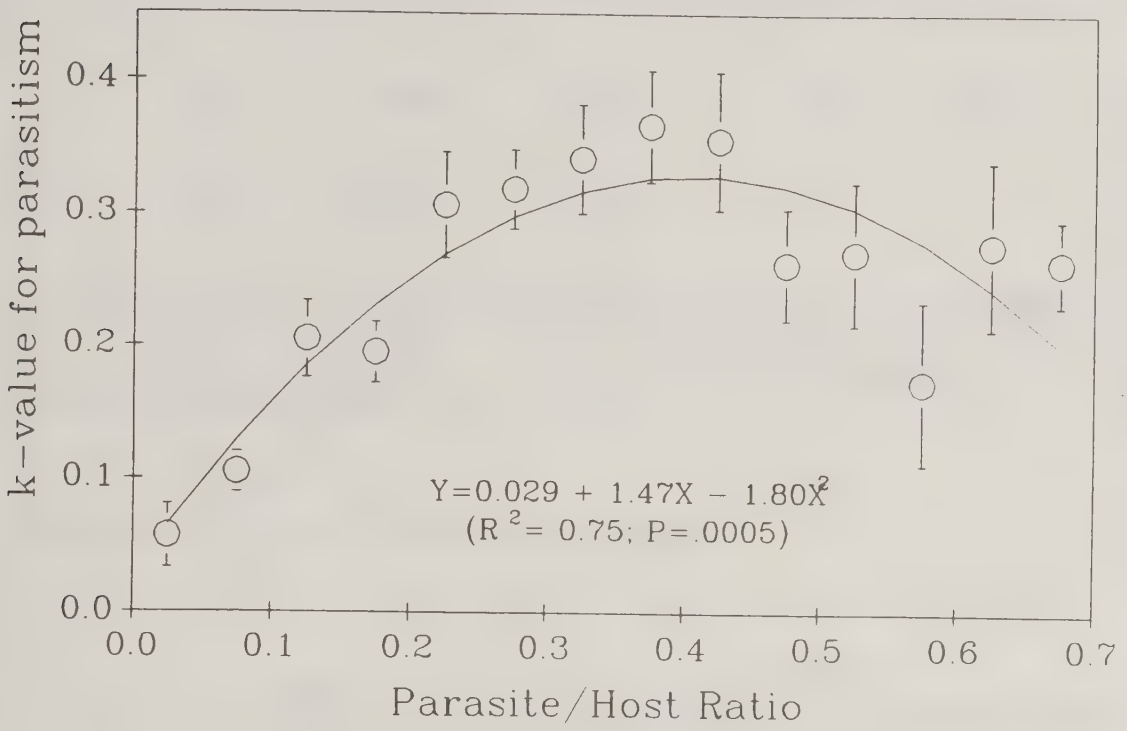


Figure 2. Influence of parasitoid female density on the number of hosts parasitized per female (Mean;SE).

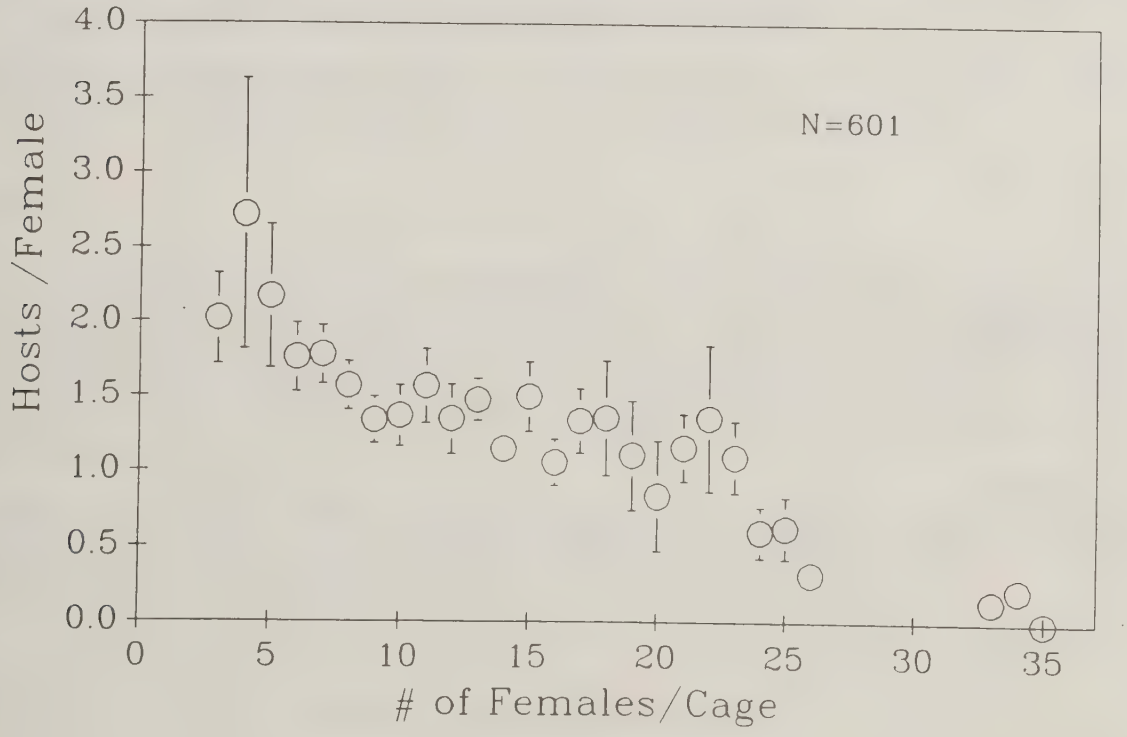


Figure 3. Area of Discovery (a) by Parasitoid Density (P) (Mean, SE).

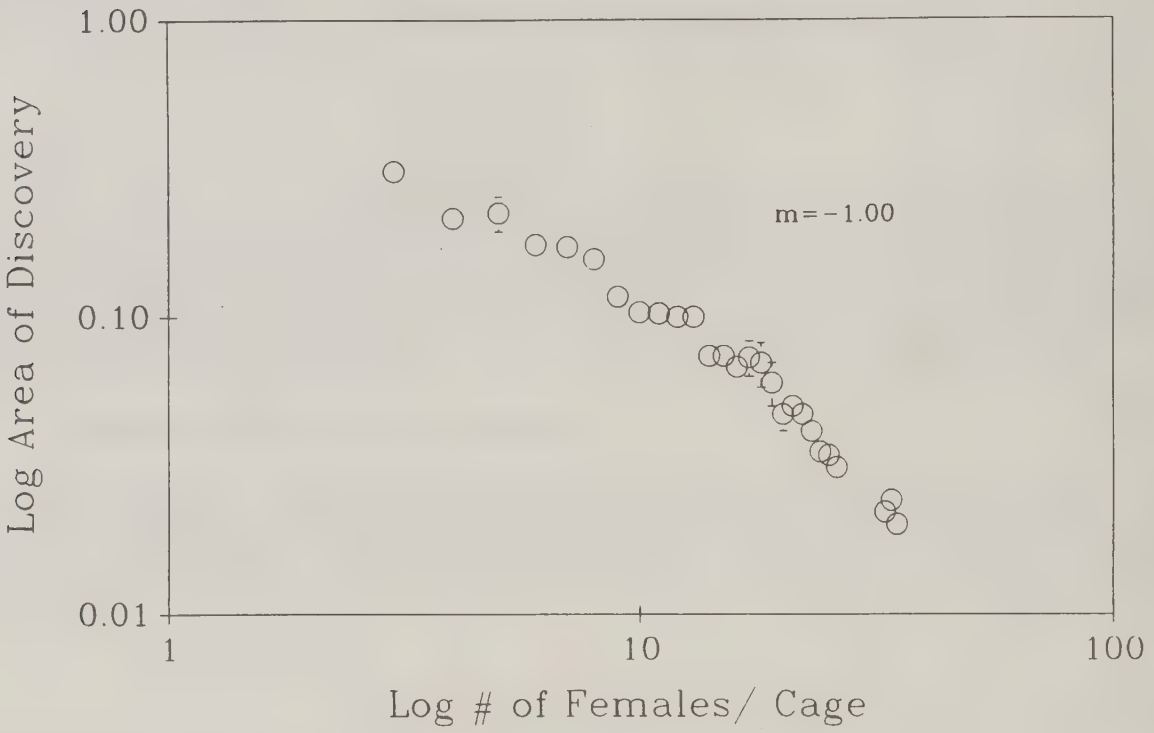
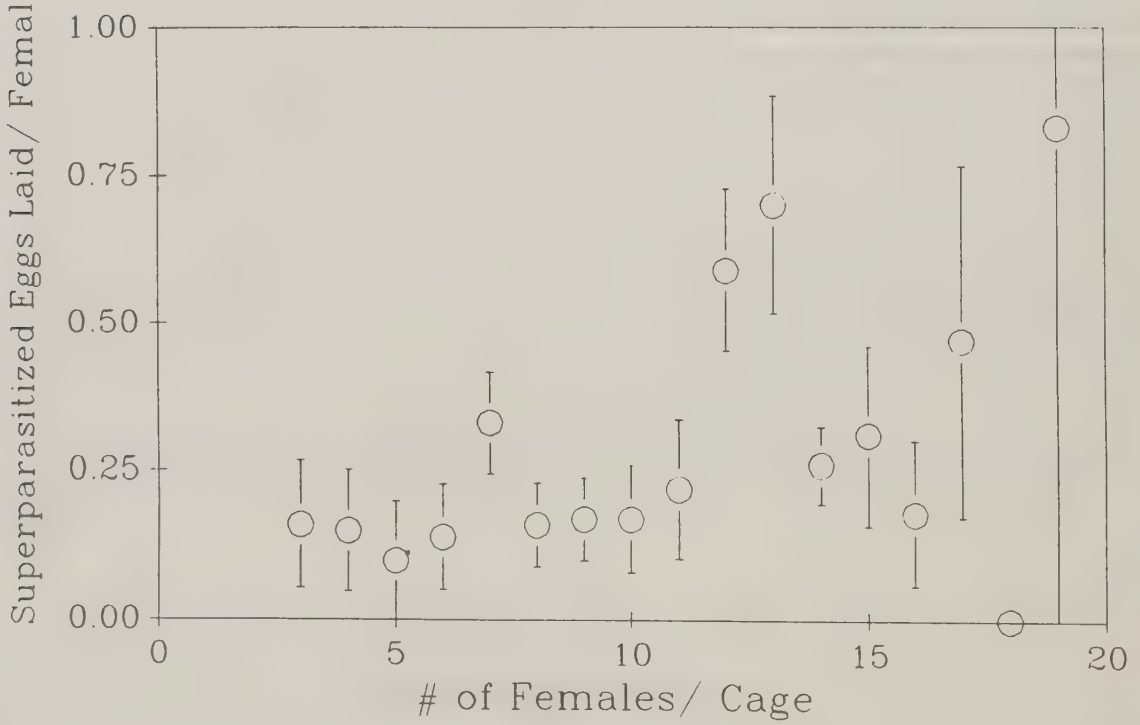


Figure 4. Number of superparasitized eggs laid per female by parasitoid density (Mean, SE).



Results and Discussion

There were no significant differences between parasitism rates when host eggs were removed from, or left on the leaf.

	N	Mean	Std Dev	T	Prob>T
Eggs on Leaf	164	49.2%	0.275	-0.11	0.90
Eggs on Paper	168	48.8%	0.327		

P/H ratios of approximately 0.25 (or 1 *E. puttleri* /4 CPB eggs) produced the highest parasitism rates when all data from both tests were pooled (Figure 1). This approximates ratios used at the Mission Biological Control Laboratory. For comparison the proportion of hosts parasitized were measured in terms of *k*-values, as described by Varley et. al. (p. 61), and were calculated as:

$$k\text{-value for parasitism} = \log N/S$$

where N = the number of hosts and S = the number not parasitized.

AGE

The overall fecundity of all female wasps in an oviposition cage is subject to some extent by the age of the parasitoid. An age specific fecundity can be calculated if one knows the individual egg production for each female. In our study this relationship between age and fecundity was not as clearly defined as that reported by Lashomb et al. Clearly, this was because females in their study were reared individually. Average rates of parasitism per *E. puttleri* female was 1.5 (SE=0.057, N=601) less than half that recorded by Lashomb et al. (ca. 5).

Infertility among females can be another factor leading to lower average fecundity rates. In our study twenty percent of 15 females dissected at the conclusion of the second trial were infertile (i.e. undeveloped ovaries). Again infertility may be affected by the increased proximity of other females.

Interestingly, average overall parasitism rates per cage of 39.7% (SE=0.012, N=601), in spite of low fecundity, were similar to the maximal percentage of 43% recorded by Lashomb et al.

INTERFERENCE

The influence of density on fecundity can be seen when the number of females per cage is plotted against the average number of hosts parasitized per female (Figure 2). Regardless of their age or the number of hosts per cage, fecundity declined with density. Clearly, mutual interference was a driving factor in the production efficiency of each female (Figure 3). Here, searching efficiency (a) declined dramatically as parasite density increased. This figure also epitomizes a parasitoid with a high mutual interference constant; ie. a steep slope (m) of 1.0.

Superparasitism, as measured by dissecting eggs in the second trial, was also related to parasite density (Figure 4). Up to five *E. puttleri* eggs were found in a single CPB egg, indicating that the marking of hosts by the females may not be as important as simple physical antagonism.

Conclusions

With an average fecundity of 1.5 eggs per day the present P/H ratios of 1/4 to 1/2 are apparently reasonable. What should be explored further are methods to increase the fecundity of each female under the conditions of our oviposition cages. This might include an increased area between egg masses as a way to reduce interference between females. Perhaps much lower parasite/host ratios are possible if we can approach the maximal fecundity of each female.

References Cited

Hassell M. P. and G. C . Varley. 1969. New inductive population model for insect parasites and its bearing on biological control. Nature, Lond., 223: 1133-1137.

Lashomb, J.,D. Krainacker, R. K. Jansson, Y. S Ng, and R. Chianese. 1987. Parasitism of *Leptinotarsa decemlineata* (Say) eggs by *Edovum puttleri* Grissell (Hymenoptera: Eulophidae): effects of host age, parasitoid age, and temperature. Can. Ent. 119: 75-82

Nicholson A. J. and V. A. Bailey. 1935. The balance of animal populations. Part I. Proc. Zool. Soc. Lond., 1935, 551-598.

Varley C. G., G. R. Gradwell and M. P. Hassell. 1973. Insect Population Ecology. University of California Press., L. A. Calif.

Project Number:	GM 78.1.3
Project Title:	Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leaders:	W. H. McLane and J. A. Finney

The objectives of this laboratory screening project are to collect and evaluate mortality data on experimental and registered compounds potentially useful for gypsy moth control, and to select materials for field studies and further development. These tests are designed to identify new materials and to increase the effectiveness of registered products.

Our main emphasis is development of new and registered materials that may improve treatments of gypsy moth in isolated infestations.

Unless otherwise stated, all tests have been conducted with our standard red oak seedling technique. Test insects are of the New Jersey strain and have been laboratory reared on artificial diet.

Five tender oak seedlings are treated with each test sample and allowed to dry for 3 hours. Twenty newly moulted, 2nd-instar gypsy moth larvae are then introduced onto each plant. Plants with test insects are then hold in an environmental chamber at 76°F and 55% RH. Insect mortality and seedling defoliation are recorded over a period of time. Five untreated seedlings are used as a control.

Materials are also tested for their stability when exposed to rainfall and ultraviolet light. Approximately 3 hours following treatment, plants are exposed to rainfall and/or ultraviolet light. They are then dried under a fan if necessary, and a standard bioassay determines effects.

During the past few years the majority of our laboratory work has been directed to the development of more efficacious *Bacillus thuringiensis* formulations. This work continued during this reporting period.

A new *Bt* carrier was tested and found to be very active with most formulations. A number of laboratory tests were conducted with the material and a limited outdoor residue test.

The data in Tables 1-2 are a result of receiving ABG-7022 with the active *Bt* already incorporated into the formulation. In all other tests the *Bt* was added to the ABG-7022 in the laboratory just before testing.

Table 1. Percent larval mortality and seedling defoliation following a 4-day exposure to seedlings treated with various *Bt* formulations.

Material	Dosage/rate BIU/gal/acre	Percent mortality	Percent defoliation
ABG-7022 (old)	2	31	38
ABG-7022 (old)	2	4	70
ABG-7022 (old)	2	10	40
H. Yield	12	22	36
H. Yield	2	1	98
Dipel 8L	12	16	48
ABG-6222	12	8	74
ABG-6223	12	25	42
ABG-6228	12	24	36
Control	-	0	100

Table 2. Percent larval mortality and seedling defoliation following exposure to seedlings treated with 3 *Bt* formulations at 2 BIU/gallon/acre then exposed to outside conditions.

Material	Days aged outside	Percent mortality			Percent defoliation		
		2 days	3 days	4 days	2 days	3 days	4 days
ABG-7022 (old)	0	17	36	52	4	16	17
	2	2	-	48	18	-	38
ABG-7022 (new)	0	7	10	11	27	46	58
	2	0	-	4	30	-	86
	4	0	-	1	50	-	71
Dipel 8AF	0	0	2	3	36	43	52
	2	0	-	19	32	-	76
	4	0	-	0	67	-	81
Control	0	0	0	0	70	90	96
	2	0	-	0	48	-	100
	4	0	-	0	88	-	99

Dipel 2x powder was mixed with ABG-7022 and tested on oak seedlings at 3 dosages.

Registered *Bt* formulations were used as a standard. Five untreated plants were used as a control. When seedlings were completely defoliated, larvae were put on artificial diet.

Table 3. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 3-4 and 7-day exposure to oak seedlings treated with *Bt*.

Material	BIU gal/acre	Percent mortality			Percent defoliation		
		3 days	4 days	7 days	3 days	4 days	7 days
Dipel 2x + ABG-7022	14.52	82	90	99	2	2	4
Dipel 2x + H_2O	14.52	22	27	64	41	49	65
Foray 48B	12.52	80	88	100	2	7	7
Thuricide 32LV	14.52	69	89	100	2	2	2
Dipel 8L	14.52	11	26	73	42	54	59
Dipel 2x + ABG-7022	7.26	95	98	100	2	2	2
Dipel 2x + H_2O	7.26	2	15	40	50	62	100
Foray 48B	7.26	45	53	99	15	20	21
Thuricide 32LV	7.26	26	62	99	3	5	5
Dipel 8L	7.26	3	6	19	62	76	100
Dipel 2x + ABG-7022	3.63	57	71	85	8	13	20
Dipel 2x + H_2O	3.63	0	0	4	70	88	100
Foray 48B	3.63	30	58	94	16	26	31
Thuricide 32LV	3.63	24	62	100	4	5	5
Dipel 8L	3.63	4	6	23	54	64	66
Control ABG-7022	--	0	0	3	84	90	100
Control -	--	0	0	0	88	94	100

After final mixes were refrigerated for 8 days, the first test (Table 4) was repeated.

Table 4. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to oak seedlings treated with Bt.

Material	BIU gal/acre	Percent mortality				Percent defoliation	
		2 days	4 days	6 days	10 days	2 days	4 days
Dipel 2x + ABG-7022	14.52	73	97	100		1	1
Dipel 2x + H_20	14.52	4	11	68	99	30	40
Foray 48B	14.52	38	79	100		3	6
Thuricide 32LV	14.52	31	75	97	99	3	7
Dipel 8L	14.52	10	20	83	97	20	30
Dipel 2x + ABG-7022	7.26	37	47	90	98	7	13
Dipel 2x + H_20	7.26	2	4	17	35	34	52
Foray 48B	7.26	19	50	90	94	5	8
Thuricide 32LV	7.26	10	42	97		13	14
Dipel 8L	7.26	0	1	3	19	44	75
Dipel 2x + ABG-7022	3.63	38	65	96	99	5	14
Dipel 2x + H_20	3.63	0	3	14	27	46	100
Foray 48B	3.63	11	31	95	100	18	30
Thuricide 32LV	3.63	14	67	94	99	9	9
Dipel 8L	3.63	0	3	13	35	48	100
Control ABG-7022	-	1	1	2	2	58	100
Control		0	0	1	2	74	100

In test 3 (Table 5) materials were placed onto oak foliage with a brush so all foliage was completely saturated with each formulation. This was a poor test technique as ABG-7022 did not dry completely and very heavy mortality occurred as a result of larvae being submersed in the carrier.

Table 5. Percent mortality of 2nd-instar gypsy moth larvae following a 2 and 4 day exposure to oak seedlings treated with Bt applied by brush.

Material	BIU gal/sol	Percent mortality 2 days	Percent mortality 4 days	Percent defoliation 2 days	Percent defoliation 4 days
Dipel 2x + ABG-7022	14.52	100	100	0	0
	7.26	95	100	0	0
	3.63	93	100	0	0
Dipel 2x + H_20	14.52	93	100	1	1
	7.26	88	100	1	1
	3.63	65	97	2	3
Foray 48B	14.52	96	100	1	1
	7.26	81	99	1	1
	3.63	65	99	1	2
Control ABG-7022	Carrier	90	100	0	0
Control	--	0	0	74	92

In the following tables, ABG-7022 (E) is the emulsified formulation.

Table 6. Percent larval mortality and seedling defoliation following exposure to oak seedlings treated with *Bt* formulations at 4 BIU/gallon/acre.

Material	Percent mortality 2 day	Percent mortality 4 day	Percent mortality 6 day	Percent defoliation 2 day	Percent defoliation 4 day	Percent defoliation 6 day
ABG-6158A	1	5	31	32	64	*
ABG-6192A	10	24	53	20	41	*
ABG-6167A	18	39	71	8	21	*
ABG-6167	21	53	94	9	17	18
ABG-6158F	1	5	20	34	62	*
ABG-6192B	0	4	16	48	84	*
ABG-6158B	2	6	30	26	74	*
ABG-6281A	19	45	89	5	19	20
ABG-6158D	2	4	31	36	68	*
ABG-6158 (752)	6	18	50	20	46	*
ABG-6158 (753)	3	11	25	21	56	*
ABG-6158 (754)	10	16	58	19	40	*
ABG-6222	5	10	41	32	64	*
ABG-6222D	1	4	23	40	76	*
ABG-6282	23	64	98	6	18	18
Foray 48B	38	80	94	11	20	23
THURICIDE 48LV	22	41	74	9	34	*
ABG-7022[1/]	48	87	96	2	2	3
ABG-7022 (E)[1/]	68	91	99	1	2	2
Control	0	0	1	85	*	*

1/ Used with Dipel 2X (powder) furnished by FMC.
* Changed to diet

A number of *Bt* formulations were compared to the ABG-7022 formulation. ABG-7022 was used with Dipel 2X powder furnished by FMC with Dipel 8L and Condor OF.

Table 7. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated with *Bt* formulations at 4 and 1 BIU/gallon/acre.

Material	Percent mortality				Percent defoliation			
	2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
Dipel 8L	1	1	12	16	40	68	--[1/]	--
Dipel 8L + ABG-7022	41	87	93	97	1	3	3	6
Condor OF	18	36	43	72	13	35	--	--
Condor OF + ABG-7022	37	64	74	92	4	11	21	35
Dipel 2X + ABG-7022[2/]	9	27	45	90	9	17	37	42
Dipel 2X _ ABG-7022	45	79	96	98	1	2	3	6
Foray 48B	13	53	83	97	3	10	34	36
Thuricide 48B	15	43	74	95	9	25	50	51
ABG-7022 only	2	2	2	2	52	100	--	--
Control	0	0	0	0	92	--	--	--

1/ Changed to artificial diet after 4 days.
2/ At 1 BIU/gallon/acre; all others at 4 BIU/gallon/acre.

A standard seedling test was conducted with ABG-7022 (E) and Dipel 8L using a number of dosages. All applications were made at a gallon per acre and no water as used in the formulation. A similar test was conducted with ABG-7022 and Dipel 8AF.

Table 8. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated with and without ABG-7022 (E) and Dipel 8L (oil).

Formulation	Dosage BIU/acre	Percent mortality				Percent defoliation			
		2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
8L + ABG-7022	16	78	97	100	--	1	3	3	
8L + Water	16	17	26	44	59	10	33	56	60
8L + ABG-7022	14	75	92	97	100	2	4	6	6
8L + Water	14	1	9	40	57	14	49	55	55
8L + ABG-7022	12	68	90	99	100	2	3	4	4
8L + Water	12	4	8	43	64	18	75	78	78
8L + ABG-7022	10	71	90	95	96	3	4	7	7
8L + Water	10	1	4	39	57	19	70	79	79
Control	--	0	0	1	1	80	98	98	98

Table 9. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated with and without ABG-7022 (E) and Dipel 8L (oil).

Formulation	Dosage BIU/acre	Percent mortality				Percent defoliation			
		2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
8L + ABG-7022 (E)	16	78	97	100	--	1	3	3	
8L + Water	16	17	26	44	59	10	33	56	60
8L + ABG-7022 (E)	14	75	92	97	100	2	4	6	6
8L + Water	14	1	9	40	57	14	49	55	55
8L + ABG-7022 (E)	12	68	90	99	100	2	3	4	4
8L + Water	12	4	8	43	64	18	75	78	78
8L + ABG-7022 (E)	10	71	90	95	96	3	4	7	7
8L + Water	10	1	4	39	57	19	70	79	79
Control	--	0	0	1	1	80	98	98	98

Table 10. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated without ABG-7022 and Dipel 8AF (aqueous).

Formulation	Dosage BIU/acre	Percent mortality				Percent defoliation			
		2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
8AF + ABG-7022 (E)	16	39	83	93	100	3	4	8	8
8AF + Water	16	23	77	97	100	5	5	11	11
8AF + ABG-7022 (E)	14	36	81	95	98	4	6	6	6
8AF + Water	14	14	50	95	98	5	12	16	16
8AF + ABG-7022 (E)	12	25	59	90	94	3	8	9	1
8AF + Water	12	11	34	84	98	5	15	21	21
8AF + ABG-7022 (E)	10	38	78	89	97	4	6	9	9
8AF + Water	10	17	48	80	93	5	16	23	23
8AF + ABG-7022 (E)	8	19	56	86	96	4	8	16	18
8AF + Water	8	3	16	75	93	8	46	59	59
8AF + ABG-7022 (E)	6	29	69	81	89	5	8	13	17
8AF + Water	6	16	60	89	95	7	34	49	49
8AF + ABG-7022 (E)	4	47	70	91	95	4	6	10	15
8AF + Water	4	10	42	63	83	7	25	46	51
8AF + ABG-7022 (E)	2	41	57	85	90	4	8	14	17
8AF + Water	2	0	23	40	50	16	66	66	66
8AF + ABG-7022 (E)	1	33	49	65	97	6	14	27	42
8AF + Water	1	2	15	33	41	21	82	91	92
Control	--	0	0	2	3	66	85	85	85

A test was conducted with Dipel 8L (oil) using various amounts of ABG-7022 (E) and water. All formulations were tested at 8 BIU/gallon/acre.

Table 11. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to various *Bt* formulations.

Formulation	Percent mortality				Percent defoliation			
	2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
8L (16oz) + Water (112oz)	0	3	22	35	38	70	79	81
8L (16oz) + Water (108oz) + ABG-7022 (4oz)	0	0	24	26	34	67	84	86
8L (16oz) + Water (104oz) + ABG-7022 (8oz)	1	3	13	21	28	83	90	90
8L (16oz) + Water (96oz) + ABG-7022 (16oz)	0	0	2	4	30	88	94	94
8L (16oz) + Water (80oz) + ABG-7022 (32oz)	0	5	30	54	28	53	68	74
8L (16oz) + Water (48oz) + ABG-7022 (64oz)	50	55	82	90	3	5	16	19
8L (16oz) + Water (0oz) + ABG-7022 (112oz)	47	75	93	97	1	5	11	19
Control	0	0	3	5	86	96	96	96

A similar test as that in Table 11 was conducted using 16 BIU/gallon/acre.

Table 12. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to various *Bt* formulations.

Formulation	Percent mortality				Percent defoliation			
	2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
8L (32oz) + Water (96oz)	9	30	68	84	18	30	42	42
8L (32oz) + Water (92oz) + ABG-7022 (4oz)	4	23	61	84	22	34	52	52
8L (32oz) + Water (88oz) + ABG-7022 (8oz)	17	36	64	87	17	32	42	46
8L (32oz) + Water (80oz) + ABG-7022 (16oz)	19	43	71	93	13	24	34	36
8L (32oz) + Water (64oz) + ABG-7022 (32oz)	23	54	79	96	10	26	30	32
8L (32oz) + Water (32oz) + ABG-7022 (64oz)	45	86	96	100	6	9	13	13
8L (32oz) + Water (0oz) + ABG-7022 (96oz)	84	100	--	--	1	1	--	--
Control	0	0	0	0	70	92	100	--

Table 13. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to various *Bt* formulations.

Formulation	Dosage BIU/acre	Percent mortality 2 day	Percent mortality 6 day	Percent defoliation 2 day	Percent defoliation 6 day
8L (2oz) + Water (62oz) + ABG-7022 (64oz)	1	5	45	15	87
8L (2oz) + Water (126oz)	1	0	5	82	96
8L (4oz) + Water (60oz) + ABG-7022 (64oz)	2	5	49	13	66
8L (4oz) + Water (124oz)	2	0	17	64	96
8L (8oz) + Water (56oz) + ABG-7022 (64oz)	4	9	67	5	31
8L (8oz) + Water (120oz)	4	0	14	62	99
8L (12oz) + Water (52oz) + ABG-7022 (64oz)	6	19	87	3	17
8L (12oz) + Water (116oz)	6	1	19	39	94
8L (16oz) + Water (48oz) + ABG-7022 (64oz)	8	31	95*	3	12*
8L (16oz) + Water (112oz)	8	0	38*	15	98*
8L (20oz) + Water (44oz) + ABG-7022 (64oz)	10	32	91*	4	18*
8L (20oz) + Water (108oz)	10	7	59*	27	85*
8L (24oz) + Water (40oz) + ABG-7022 (64oz)	12	43	99*	2	5*
8L (24oz) + Water (104oz)	12	1	62*	12	72*
8L (28oz) + Water (36oz) + ABG-7022 (64oz)	14	43	98*	3	8*
8L (28oz) + Water (100oz)	14	1	49*	17	85*
8L (32oz) + Water (32oz) + ABG-7022 (64oz)	16	46	94*	3	13*
8L (32oz) + Water (96oz)	16	14	78*	17	41*
Control	--	0	1	74	94

* Reading made after exposure of 7 days.

Dipel 8L was used with ABG-7022 (E) and tested at various dosages.

Table 14. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated with various dosages of Dipel 8L and ABG-7022 (E).

Formulation	Dosage BIU/gal/acre	Percent mortality			Percent defoliation		
		2 day	4 day	8 day	2 day	4 day	8 day
8L + ABG-7022 (E)	1	42	57	75	10	20	26
8L + Water	1	0	0	2		90	100
8L + ABG-7022 (E)	2	45	69	94	5	11	12
8L + Water	2	1	1	3	77	99	99
8L + ABG-7022 (E)	4	47	78	98	3	7	8
8L + Water	4	0	1	5	64	91	91
8L + ABG-7022 (E)	6	57	85	98	2	5	6
8L + Water	6	2	2	35	27	71	73
8L + ABG-7022 (E)	8	58	84	94	3	6	12
8L + Water	8	1	3	34	36	92	92
Control	--	0	0	1	80	98	98

Condor OF was tested with ABG-7022 (E) at a number of dosages. A comparison formulation of Condor OF and water was used. The test was conducted with a rate of one gallon per acre.

Table 15. Percent larval mortality and seedling defoliation after exposure of 2nd instar gypsy moth larvae to seedlings treated with various dosages of Condor OF and ABG-7022 (E).

Formulation	Dosage BIU/acre	Percent mortality 2 day	4 day	8 day	Percent defoliation 2 day	4 day	8 day
Condor OF + ABG-7022 (E)	16	75	96	99	2	4	5
Condor OF + Water	16	14	34	100	11	26	37
Condor OF + ABG-7022 (E)	14	91	100		2	2	
Condor OF + Water	14	23	55	100	8	20	26
Condor OF + ABG-7022 (E)	12	84	98	99	2	3	5
Condor OF + Water	12	12	30	92	11	32	50
Condor OF + ABG-7022 (E)	10	69	92	98	4	4	6
Condor OF + Water	10	3	14	92	19	42	59
Condor OF + ABG-7022 (E)	8	51	67	88	8	18	30
Condor OF + Water	8	8	13	74	13	27	37
Condor OF + ABG-7022 (E)	6	57	63	82	5	9	26
Condor OF + Water	6	7	20	89	16	32	55
Condor OF + ABG-7022 (E)	4	54	76	90	4	9	21
Condor OF + Water	4	1	9	35	23	70	74
Condor OF + ABG-7022 (E)	2	41	48	70	6	14	46
Condor OF + Water	2	2	2	15	32	86	86
Condor OF + ABG-7022 (E)	1	25	34	57	14	34	73
Condor OF + Water	1	2	8	16	33	67	73
Control	--	0	0	1	78	95	95

Twenty-five tender northern red oak seedlings were treated with each of a number of *Bacillus thuringiensis* samples. Five plants of each treatment were bioassayed on the day of treatment (Day 0) with the remaining plants held outside and bioassayed over the nine day post-treatment period. Hours of natural sunlight and inches of rainfall were recorded over the period of time plants were held outside. A group of untreated plants was used as controls.

Twenty newly moulted 2nd instar, laboratory-reared gypsy moth larvae were introduced onto each plant. Plants and test insects were held in an environmental chamber at 78°F with 55% RH.

Larval mortality and seedling defoliation were recorded over a period of time. Larvae were removed from seedlings and placed onto artificial diet after 48 hours to cut down natural mortality.

Table 16. Percent mortality of 2nd instar gypsy moth larvae after exposure to oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to natural sunlight.

Material	Percent mortality											
	Day 0[1]			Day 2			Day 7			Day 9		
	4 day[2]	8 day	12 day	4 day	8 day	12 day	4 day	6 day	12 day	4 day	8 day	12 day
MYX-2284	34	92	98	43	95	100	12	17	25	0	11	17
MYX-2728	5	16	30	85	100		4	17	25	0	9	12
MYX-2728-168	18	91	99	14	97	100	0	8	24	0	3	3
MYX-7275	3	26	55	6	59	74	1	3	6	0	1	2
MYX-7275M	29	75	91	37	97	100	0	3	5	2	4	6
MYX-8242	86	100		45	99	100	2	19	24	5	17	18
Foray 48B	48	93	100	73	100		10	22	28	10	36	47
Dipel 8L	9	39	59	19	67	84	0	1	2	1	3	3
ABG-7022 + Dipel 2X	86	94	98	61	93	100	41	56	65	14	40	46
ABG-7022 + Dipel 2X + Emul	72	91	98	72	96	98	28	54	70	0	11	16
Control	0	1	2	0	2	5	0	0	1	0	0	1

1/ Days of outside exposure
2/ Days larvae exposed to plants

Table 17. Percent defoliation of oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to 2nd instar gypsy moth larvae.

	Percent defoliation							
	Day 0[1/]		Day 2		Day 7		Day 8	
Material	2 day[2/]	4 day	2 day	4 day	2 day	4 day	2 day	4 day
MYX-2284	8	32	10	15	13	26	13	50
MYX-2728	46	82	2	4	10	30	10	32
MYX-2728-168	5	16	2	5	13	35	23	45
MYX-7275	36	74	26	42	12	44	23	44
MYX-7275M	20	48	5	14	21	46	12	32
MYX-8242	8	10	3	11	7	20	21	58
Foray 48B	15	46	4	9	9	32	28	47
Dipel 8L	27	58	22	29	19	60	17	42
ABG-7022 + Dipel 2X	1	5	3	5	4	12	6	20
ABG-7022 + Dipel 2X + Emul	2	8	2	8	5	10	8	32
Control	52	80	50	90	26	50	16	42

1/ Days of outside exposure
2/ Days larvae exposed to plants

On day 4 of the outdoor exposure, .10 inches of rain was received on the seedlings.

Table 18. Average temperatures and hours sunlight during residue test.

	Average temperature °F			
Date	0800	1200	1630	Hours sunlight
8/29	Test started			
8/30	70°	82°	74°	4
8/31	64°	78°	80°	10
9/01	67°	70°	76°	0
9/02[1/]	72°	88°	84°	8
9/03	68°	76°	74°	10
9/04	64°	78°	74°	10
9/05	67°	74°	69°	5
9/06	62°	80°	76°	2
9/07	74°	80°	72°	10

1/ Rainfall 0.1 inches

A number of tests were conducted with *Bt* formulations from Abbott Laboratories.

Table 19. Percent larval mortality and seedling defoliation following exposure to oak seedlings treated with *Bt* formulations at 16 BIU/gallon/acre.

Material	Percent mortality				Percent defoliation			
	2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
ABG-6158A	13	27	74	90	22	30	38	46
ABG-6192A	7	40	67	86	20	40	52	54
ABG-6167A	22	50	93	99	5	21	21	21
ABG-6167	38	62	100		3	5	5	5
ABG-6158F	11	38	71	87	15	31	31	31
ABG-6158B	3	33	70	89	20	42	50	56
ABG-6281A	27	87	99	100	5	6	6	6
ABG-6158D	14	61	83	97	10	30	32	38
ABG-6158 (752)	10	35	67	97	4	10	18	27
ABG-6158 (753)	17	55	84	99	3	9	10	10
ABG-6158 (754)	22	71	84	99	4	7	11	11
ABG-6222	30	87	99	100	4	8	8	8
ABG-6222D	25	67	92	100	4	12	12	12
ABG-6282	44	94	100		2	2	2	2
Foray 48B	59	93	100		3	3	3	3
Control	0	0	1	1	58	88		

Table 20. Percent larval mortality and seedling defoliation following exposure to oak seedlings treated with *Bt* formulations at 16 BIU/gallon/acre and exposed to 0.10 inches of rain 3 hours after treatment.

	Percent mortality				Percent defoliation		
Material	2 day	4 day	6 day	8 day	2 day	4 day	6 day
ABG-6158A	3	6	37	46	46	70	72
ABG-6192A	8	9	39	56	28	64	76
ABG-6167A	1	5	16	22	42	62	*
ABG-6167	3	8	37	54	26	62	62
ABG-6158F	1	8	55	60	42	60	67
ABG-6192B	0	0	21	42	44	58	60
ABG-6158B	6	8	29	38	34	64	68
ABG-6281A	30	42	82	91	13	46	46
ABG-6158D	0	2	7	17	52	86	*
ABG-6222	1	1	53	60	38	80	80
ABG-6222D	1	8	15	32	32	34	*
ABG-6282	6	8	45	67	22	55	*
Foray 48B	12	26	67	93	15	40	64
Control	0	0	0	0	100	*	

* Changed to diet

Thirty tender oak seedlings were treated in a laboratory spray chamber with 12 experimental *Bt* formulations from Abbott Laboratories. A dosage and rate of 16 BIU/gallon/acre were used. Foray was included as a standard. A similar number of untreated plants was used as a control.

Five plants of each were bioassayed on the day they were treated. The remaining plants were held outdoors in the direct sunlight with complete exposure to any rainfall that might occur. Five seedlings of each were returned to the laboratory for bioassay following various exposure times.

Newly moulted 2nd instar gypsy moth larvae were exposed to each plant with the test being conducted at 78°F with 55% RH. Larvae were exposed to foliage for four days and then placed onto artificial diet. If all foliage was consumed before the four day period, larvae were placed on diet at that time to prevent mortality due to starvation.

Larval mortality and seedling defoliation were recorded over a period of time.

Table 21. Percent mortality of 2nd instar gypsy moth larvae following a 6 and 10 day exposure to oak seedlings treated with 13 *Bt* formulations at 16 BIU/gallon/ acre, then exposed to various amounts of outdoors exposure.

ABG Material	Number of days plants aged outside											
	0[1]		2		4		7		9		11	
	6[2]	10	6	10	6	10	6	10	6	10	6	10
6158A	71	92	87	97	3	3	4	14	25	36	6	20
6192A	56	69	65	79	15	25	3	10	3	9	3	11
6167	97	100	91	100	9	20	1	11	2	3	3	7
6167A	77	97	84	97	25	29	5	17	11	16	8	14
6158F	81	91	84	85	10	22	21	27	7	23	5	11
6192B	7	25	32	73	1	4	2	11	2	4	1	6
6158B	71	88	86	99	11	31	13	32	7	21	6	15
6281A	97	99	38	77	5	6	6	12	10	16	4	9
6222	88	91	86	100	32	38	19	33	27	44	10	17
6222D	77	98	76	99	17	32	21	35	13	21	9	14
6158D	88	98	79	94	10	30	4	21	6	10	3	6
6282	92	100	93	100	16	41	17	39	23	28	8	18
Foray	100	100	100	100	49	80	19	28	29	39	4	10
Control	2	6	0	1	2	9	6	9	0		2	4

1/ Number of days aged outdoors
2/ Days following initial exposure of larvae to plants

Table 22. Percent oak seedling defoliation following a 2 and 4 day exposure to gypsy moth larvae following treatment with 13 *Bt* formulations at 16 BIU/gallon/acre, then exposed to various amounts of outdoors exposure.

ABG Material	Number of days plants aged outside											
	0[1]		2		4		7		9		11	
	2[2]	4	2	4	2	4	2	4	2	4	2	4
6158A	9	22	12	18	44	84	34	80	8	27	6	40
6192A	33	54	15	23	14	42	40	74	14	42	5	32
6167	10	21	12	32	18	46	30	62	10	20	8	30
6167A	18	34	7	22	27	46	30	36	16	34	6	48
6158F	11	24	5	20	24	46	28	54	9	16	11	29
6192B	36	80	20	37	17	78	30	52	12	20	5	28
6158B	12	36	6	14	19	42	28	56	8	22	9	26
6281A	10	31	26	48	22	52	34	56	8	16	7	26
6222	13	42	16	25	16	44	27	48	13	35	4	30
6222D	22	46	9	17	15	27	24	48	10	24	5	30
6158D	6	27	7	11	12	35	32	52	6	24	7	28
6282	6	16	10	17	32	64	32	48	8	25	7	38
Foray	6	8	6	7	9	24	30	52	8	15	11	41
Control	82	100	68	100	64	98	56	72	49	68	15	42

1/ Number of days aged outdoors
2/ Days following initial exposure of larvae to plants

Hours of natural sunlight per day =

Day	Hours	Day	Hours
1	10	7	10
2	10	8	2
3	8	9	0
4	10	10	4
5	10	11	10
6	10		

The average temperature was =

Time	Temp.
8:00 AM	73
12:30 PM	79
4:00 PM	76

Rainfall =

Day	Amount
9	.15 inches
10	.70 inches

Table 23. Percent mortality of 2nd instar gypsy moth larvae following a 6 and 10 day exposure to oak seedlings treated with 7 *Bt* formulations at 16 BIU/gallon/acre, then expose to various amounts of outdoor exposure.

	Number of days plants aged outside													
	0[1]		2		4		7		9		11		14	
Material	6[2]	10	6	10	6	10	6	10	6	10	6	10	6	10
Dipel 8L	9	31	40	87	12	81	10	82	4	62	9	62	3	48
Dipel 8AF	84	100	65	98	38	93	38	77	5	22	16	60	24	95
Foray 48B	91	100	85	99	73	93	84	98	48	92	66	98	65	100
Condor OF	91	100	56	89	35	72	45	89	9	65	58	93	33	85
Condor AF	93	100	69	93	52	89	46	98	2	77	43	92	11	72
SAN-415	96	99	79	100	52	97	67	90	17	70	10	80	12	72
Thuricide 32LV	93	100	81	100	72	98	53	90	17	82	16	51	30	92
Control	2	6	0	7	0	7	2	32	1	18	16	65	1	41

1/ Number of days aged outdoors
2/ Days following initial exposure of larvae to plants

The 10 day mortality after 7 days of aging outdoors is partly due to natural death as is indicated in the control. Mortality has not been corrected by "Abbott's Formula".

Table 24. Percent oak seedling defoliation following a 2 and 4 day exposure to gypsy moth larvae following treatment with 7 *Bt* formulations at 16 BIU/gallon/acre, then exposed to various amounts of outdoors exposure.

	Number of days plants aged outside													
	0[1/]		2		4		7		9		11		14	
Material	2[2/]	4	2	4	2	4	2	4	2	4	2	4	2	4
Dipel 8L	50	72	32	41	26	32	19	32	18	23	4	34	20	44
Dipel 8AF	8	28	26	37	19	30	20	30	28	49	9	36	10	24
Foray 48B	6	16	10	15	22	31	14	25	16	19	4	11	12	22
Condor OF	7	23	30	40	41	52	18	38	24	30	2	24	11	23
Condor AF	7	19	20	26	19	24	8	15	22	27	4	22	17	32
SAN-415	5	13	16	17	21	26	9	20	19	19	5	26	9	30
Thuricide 32LV	4	14	20	20	22	23	20	30	16	17	7	35	10	20
Control	66	70	38	74	50	76	28	42	22	26	9	36	26	58

1/ Number of days aged outdoors
2/ Days following initial exposure of larvae to plants

The amount of natural rain recorded was =

Day	Inches
8	.65
11	.10
11	1.10

Hours of natural sunlight per day =

Day	Hours	Day	Hours
1	2	8	0
2	3	9	10
3	7	10	10
4	6	11	7
5	2	12	0
6	4	13	6
7	6	14	0

The average temperature was =

Time	Temp.
8:00 AM	72
12:30 PM	86
4:00 PM	82

A weathering test was conducted with Dipel 8L, Dipel 8AF and Foray. Plants were treated and then dried for 3 hours before exposure to rainfall.

Table 25. Percent larval mortality and seedling defoliation following exposure to seedlings treated with *Bt* at 20 BIU/gallon/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality			Percent defoliation		
		2 days	4 days	8 days	2 days	4 days	8 days
Dipel 8L	--	9	22	64	10	42	--[1/]
"	.10	2	8	31	21	58	--
"	.25	2	2	10	40	88	--
Dipel 8AF	--	47	86	100	3	6	6
"	.10	11	34	88	19	38	--
"	.25	2	27	69	10	35	--
Foray 48B	--	61	90	100	4	5	5
"	.10	2	14	45	12	50	--
"	.25	0	2	26	15	58	--
Control	--	0	1	3	75	100	--
"	.25	0	0	0	81	100	--

1/ changed to artificial diet after 4 days

A number of new stickers were tested with Dipel 8L using 10.6 BIU/gallon/acre. Each treatment was dried for 2 hours before being exposed to rainfall.

Table 26. Percent larval mortality and seedling defoliation following exposure to seedlings treated with Dipel 8L and various stickers and exposed to rainfall.

Sticker	Inches rain	Percent mortality			Percent defoliation		
		4 days	6 days	10 days	2 days	4 days	6 days
--	--	9	36	58	24	52	59
--	1.0	0	3	10	76	87	100
Bond 1%	--	13	51	80	18	44	50
"	1.0	1	8	18	41	73	77
Bond 2%	--	8	33	75	20	38	44
"	1.0	1	6	21	38	40	58
Bond 3%	--	22	65	96	17	25	30
"	1.0	1	7	14	37	62	70
Biofilm 1%	--	24	80	93	19	30	32
"	1.0	1	4	13	32	60	-- [1/]
Biofilm 2%	--	24	61	98	24	30	46
"	1.0	3	14	28	34	60	62
Biofilm 3%	--	35	64	88	11	30	42
"	1.0	3	23	35	40	64	--
Sprayfuse 1%	--	1	24	43	32	62	--
"	1.0	1	4	12	40	52	--
Sprayfuse 2%	--	10	30	58	36	46	53
"	1.0	0	2	8	74	94	--
Sprayfuse 3%	--	11	40	67	27	54	61
"	1.0	0	0	2	68	100	--
Surfix 1%	--	16	38	71	12	48	48
"	1.0	0	4	7	29	74	--
Surfix 2%	--	20	71	89	7	40	42
"	1.0	0	2	5	43	72	--
Surfix 3%	--	11	47	77	14	54	--
"	1.0	1	4	6	39	86	94
Control	--	1	4	9	51	83	--
"	1.0	1	2	8	70	95	--

1/ changed to artificial diet after 4 days

Table 27. Percent larval mortality and seedling defoliation following exposure to seedlings treated with Dipel 8L at 16 BIU/gallon/acre and various stickers and exposed to rainfall.

Sticker	Inches rain	Percent mortality			Percent defoliation		
		4 days	6 days	10 days	2 days	4 days	6 days
--	--	8	29	56	23	64	-- 1/
--	1.0	0	1	2	80	96	--
Bond 1%	1.0	1	1	1	46	82	--
Bond 2%	--	8	32	62	24	69	--
"	1.0	0	7	14	60	82	85
Bond 3%	1.0	0	3	11	44	76	--
Blue Ribbon 1%	--	20	47	70	32	51	62
"	1.0	1	8	10	54	79	87
Blue Ribbon 2%	--	12	35	61	35	57	65
"	1.0	1	3	6	74	--	--
Blue Ribbon 3%	--	25	49	72	26	51	68
"	1.0	1	1	1	74	92	--
Chem-Stik 2%	--	4	39	67	15	51	57
"	1.0	2	23	39	36	78	87
Target NL 2%	--	5	20	53	19	66	78
"	1.0	0	1	5	84	99	--
Surfix 2%	--	12	44	67	35	80	83
"	1.0	0	2	7	74	92	--
Poly AG 2%	--	32	70	99	14	46	53
"	1.0	0	6	32	31	63	71
Control	--	2	3	6	82	--	--
"	1.0	1	1	2	71	--	--

1/ changed to artificial diet after 4 days

Dipel 8AF and Foray 48B were used to test our laboratory strain of gypsy moth against wild insects from Virginia and West Virginia. This test was to assure that our laboratory test strain of gypsy moth larvae is giving the same results as wild strains.

Table 28. Percent larval mortality and seedling defoliation following exposure to seedlings treated with *Bt* at 16 BIU/gallon/acre.

		Percent mortality				Percent defoliation		
Material	Strain	2 days	4 days	6 days	8 days	2 days	4 days	8 days
Dipel 8AF	Lab	7	22	64	90	7	32	34
"	VA	6	26	60	81	5	15	28
"	WV	14	36	71	90	5	10	27
Foray 48B	Lab	26	63	91	100	5	18	20
"	VA	11	56	80	95	2	5	12
"	WV	25	75	88	99	1	3	6
Control	Lab	0	0	0	3	68	88	--[1/]
"	VA	0	2	3	17	56	100	--
"	WV	0	5	18	37	36	100	--

1/ changed to artificial diet after 4 days

During this reporting period a number of experimental *Bt* formulations were tested for Mycogen Corporation.

Table 29. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to oak seedlings treated with a number of *Bacillus thuringiensis* formulations.

Material	BIU gal/acre	Percent mortality					Percent defoliation
		2 days	4 days	6 days	8 days	11 days	2 days
MYX-2284	2	1	1	10	13	17	60
MYX-2284	4	0	0	16	26	32	40
MYX-8018	2	0	1	5	23	56	48
MYX-8018	4	1	2	19	62	94	38
MYX-7275	2	0	2	14	30	36	54
MYX-2725	4	0	0	9	45	71	36
Foray 48B	2	10	35	72	67	78	18
Foray 48B	4	22	51	80	83	87	12
Thuricide 32LV	2	9	21	71	72	76	6
Thuricide 32LV	4	6	23	79	82	85	5
Dipel 8L	2	1	1	4	6	7	70
Dipel 8L	4	0	2	10	14	22	52
Control	-	0	1	2	2	3	84

* All test insects changed to diet after 2 days exposure to treated foliage.

Table 30. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to oak seedlings treated with *Bacillus thuringiensis* at 8 BIU/gal/acre.

Material	Percent mortality				Percent defoliation			
	2 days	4 days	8 days	10 days	1 day	2 days	4 days	6 days
MYX-2284	2	15	68	73	5	16	50	54
MYX-8018	2	15	82	89	5	30	52	54
MYX-7275	1	21	83	92	5	21	47	56
Foray 48B	30	94	100		3	5	9	9
Thuricide 32LV	24	95	100		2	2	2	2
Dipel 8AF	17	51	92	97	3	7	25	27
Dipel 8L	1	1	58	65	9	42	84	84
Control	0	0	0	0		35	82	100

* All test insects changed to diet after 6 days exposure to treated foliage.

Table 31. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following exposure to oak seedlings treated with *Bacillus thuringiensis* at 12 BIU/gal/acre.

Material	Percent mortality				Percent defoliation		
	2 days	4 days	8 days	10 days	2 days	4 days	6 days
MYX-2284	32	60	100		4	5	5
MYX-8018	4	23	85	93	15	32	36
MYX-7275	1	18	78	88	25	39	42
Foray 48B	48	90	100		4	8	8
Dipel 8L[1/]	1	7	34	45	32	62	
Control[1/]	0	0	1	1	80	100	

1/ Changed to diet after 4 days on foliage.

Table 32. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following exposure to oak seedlings treated with *Bacillus thuringiensis* at 12 BIU/gal/acre.

Material	Percent mortality				Percent defoliation		
	2 days	4 days	8 days	10 days	1 day	2 days	4 days
MYX-2284	26	59	96	100	4	8	14
MYX-8018	1	13	52	77	6	26	44
MYX-7275	0	6	27	37	9	44	50
Foray 48B	19	86	100		3	3	10
Thuricide 32LV	27	75	100		2	4	5
Dipel 8AF	6	22	79	95	5	8	22
Dipel 8L	3	8	42	50	6	30	56
Control	0	0	1	1	47	86	100

Table 33. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following exposure to oak seedlings treated with *Bacillus thuringiensis* at 14 BIU/gal/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality				Percent defoliation	
		2 days	4 days	8 days	10 days	2 days	4 days
MYX-2284	--	56	95	100	100	6	6
MYX-2284	.5	1	2	48	50	25	49
MYX-8018	--	5	36	92	99	14	30
MYX-8018	.5	0	0	13	15	40	79
MYX-7275	--	0	9	67	84	25	57
MYX-7275	.5	0	0	6	7	78	100
Foray 48B	--	72	94	100	100	5	5
Foray 48B	.5	2	8	69	71	19	52
Dipel 8AF	--	3	73	96	100	6	7
Dipel 8AF	.5	6	22	69	71	24	61
Dipel 8L	--	58	78	98	100	2	3
Dipel 8L	.5	1	4	22	29	63	93
Control	--	0	0	3	4	81	100

* Treated plants exposed to rainfall approximately 3 hours after treatment was applied.

Twenty-five tender northern red oak seedlings were treated with each of a number of *Bacillus thuringiensis* samples. Five plants of each treatment were bioassayed on the day of treatment (Day 0) with the remaining plants held outside and bioassayed over the nine-day post-treatment period. Hours of natural sunlight and inches of rainfall were recorded over the period of time plants were held outside. A group of untreated plants was used as controls.

Twenty newly moulted 2nd instar, laboratory-reared gypsy moth larvae were introduced onto each plant. Plants and test insects were held in an environmental chamber at 78°F with 55% RH.

Larval mortality and seedling defoliation were recorded over a period of time. Larvae were removed from seedlings and placed onto artificial diet after 48 hours to cut down on natural mortality.

Table 34. Percent mortality of 2nd instar gypsy moth larvae after exposure to oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to natural sunlight and rainfall.

	Percent mortality											
	Day 0[1/]			Day 2			Day 7			Day 9		
Material	4 day[2/]	8 day	12 day	4 day	8 day	12 day	4 day	6 day	12 day	4 day	8 day	12 day
MYX-2284	34	92	98	43	95	100	12	17	25	0	11	17
MYX-2728	5	16	30	85	100		4	17	25	0	9	12
MYX-2728-168	18	91	99	14	97	100	0	8	24	0	3	3
MYX-7275	3	26	55	6	59	74	1	3	6	0	1	2
MYX-7275M	29	75	91	37	97	100	0	3	5	2	4	6
MYX-8242	86	100		45	99	100	2	19	24	5	17	18
FORAY 48B	48	93	100	73	100		10	22	28	10	36	47
Dipel 8L	9	39	59	19	67	84	0	1	2	1	3	3
ABG-7022 + Dipel 2X	86	94	98	61	93	100	41	56	65	14	40	46
ABG-7022 + Dipel 2X + Emul	72	91	98	72	96	98	28	54	70	0	11	16
Control	0	1	2	0	2	5	0	0	1	0	0	1

1/ Days of outside exposure
2/ Days larvae exposed to plants

Table 35. Percent defoliation of oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to 2nd instar gypsy moth larvae.

Material	Percent defoliation							
	Day 0[1/]		Day 2		Day 7		Day 8	
	2 day[2/]	4 day	2 day	4 day	2 day	4 day	2 day	4 day
MYX-2284	8	32	10	15	13	26	13	50
MYX-2728	46	82	2	4	10	30	10	32
MYX-2728-168	5	16	2	5	13	35	23	45
MYX-7275	36	74	26	42	12	44	23	44
MYX-7275M	20	48	5	14	21	46	12	32
MYX-8242	8	10	3	11	7	20	21	58
Foray 48B	15	46	4	9	9	32	28	47
Dipel 8L	27	58	22	29	19	60	17	42
ABG-7022 + Dipel 2X	1	5	3	5	4	12	6	20
ABG-7022 + Dipel 2X + Emul	2	8	2	8	5	10	8	32
Control	52	80	50	90	26	50	16	42

1/ Days of outside exposure
2/ Days larvae exposed to plants

On day 4 of the outdoor exposure, .10 inch of rain was received on the seedlings.

Table 36. Average temperatures and hours sunlight during residue test.

Date	Average temperature °F			Hours sunlight
	0800	1200	1630	
8/29	Test started			
8/30	70°	82°	74°	4
8/31	64°	78°	80°	10
9/01	67°	70°	76°	0
9/02[1/]	72°	88°	84°	8
9/03	68°	76°	74°	10
9/04	64°	78°	74°	10
9/05	67°	74°	69°	5
9/06	62°	80°	76°	2
9/07	74°	80°	72°	10

1/ Rainfall 0.1 inches

Tests were conducted with E-2492, a *Bt* formulation from C.I.L., Inc.

Table 37. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with E-2492 and standard *Bacillus thuringiensis* formulations.

Material	Dosage BIU/gal/acre	Percent mortality					Percent defoliation			
		2 day	4 day	6 day	8 day	10 day	2 day	4 day	6 day	8 day
E-2492	4	4	8	28	45	57	28	76		
"	8	4	11	54	72	80	19	71		
"	12	11	41	88	95	100	15	29	38	59
"	16	16	48	90	97	100	17	35	42	59
"	20	25	65	93	97	100	7	14	17	29
Foray 47B	4	9	34	87	99	99	30	44	52	52
"	8	23	53	92	96	99	7	20	22	22
"	12	27	75	99	99	100	14	14	18	18
"	16	13	63	93	99	99	12	19	22	22
"	20	37	83	100			4	8	8	8
Condor	4	0	0	1	4	9	50			
"	8	1	3	23	31	44	40			
"	12	9	23	75	88	95	30			
"	16	19	35	61	81	88	25	33	66	
"	20	20	47	70	90	97	19	26	82	
Dipel 8L	4	1	1	7	23	56	36	82		
"	8	0	2	9	10	12	42			
"	12	3	4	23	38	53	26	83		
"	16	2	17	62	79	93	22	68	70	90
"	20	5	14	41	72	89	22	52	78	
Dipel 8AF	4	9	23	50	70	85	28	59	73	
"	8	17	60	85	95	99	17	46	52	
"	12	28	62	86	98	99	8	16	23	
"	16	26	93	100			4	12	13	
"	20	27	86	99	100		5	10	10	
Control	--	0	1	1	3	3	68	100		

Table 38. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with E-2492 and standard *Bacillus thuringiensis* formulations.

Material	Dosage BIU/gal/acre	Percent mortality				Percent defoliation			
		2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
E-2492	4	1	14	56	77	14	49	76	
"	8	5	19	64	85	12	40	43	47
"	12	22	41	82	96	9	21	33	36
"	16	17	38	73	96	6	36	44	
"	20	6	29	78	98	5	17	24	25
Thuricide 32LV	4	8	44	89	99	5	10	15	17
"	8	4	33	78	97	5	6	8	8
"	12	24	52	84	95	5	10	10	10
"	16	12	64	96	100	3	11	12	12
"	20	21	63	97	99	2	3	3	3
SAN 415-NRD 12	4	4	12	73	90	8	17	42	42
"	8	8	48	90	100	7	10	16	16
"	12	2	22	79	95	10	14	29	32
"	16	14	57	94	100	5	6	9	9
"	20	18	62	98	100	2	3	5	5
Control	--	0	0	0	1	82	96		

Table 39. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with E-2492 and standard *Bacillus thuringiensis* formulations at 20 BIU/gallon/acre and exposed to rainfall.

Material	Inches rain	Percent mortality		Percent defoliation	
		2 day	4 day	2 day	4 day
E-2492	--	31	79	5	8
"	0.1	12	35	8	42
"	0.25	2	10	15	62
Foray 48B	--	61	90	3	3
"	0.1	2	14	12	50
"	0.25	0	2	15	58
Dipel 8L	--	9	22	10	42
"	0.1	2	8	21	58
"	0.25	2	2	40	88
Dipel 8AF	--	47	86	3	6
"	0.1	11	34	19	38
"	0.25	2	27	10	35
Control	--	0	1	75	100
"	0.25	0	0	81	100

Twenty-five tender northern red oak seedlings were treated with each of a number of *Bacillus thuringiensis* samples. Five plants of each treatment were bioassayed on the day of treatment (Day 0) with the remaining plants held outside and bioassayed over the nine-day post-treatment period. Hours of natural sunlight and inches of rainfall were recorded over the period of time plants were held outside. A group of untreated plants was used as controls.

Twenty newly moulted 2nd instar, laboratory-reared gypsy moth larvae were introduced onto each plant. Plants and test insects were held in an environmental chamber at 78°F with 55% RH.

Larval mortality and seedling defoliation were recorded over a period of time. Larvae were removed from seedlings and placed onto artificial diet after 48 hours to cut down on natural mortality.

Table 40. Percent mortality of 2nd instar gypsy moth larvae after exposure to oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to natural sunlight and rainfall.

	Percent mortality											
	Day 0[1/]			Day 2			Day 7			Day 9		
Material	4 day[2/]	8 day	12 day	4 day	8 day	12 day	4 day	6 day	12 day	4 day	8 day	12 day
E2492	5	40	79	7	91	94	0	2	3	0	0	1
E2492 UV	25	69	93	6	73	88	0	0	3	0	2	3
Dipel 8L	9	39	59	19	67	84	0	1	2	1	3	3
Foray 48B	48	93	100	73	100		10	22	28	10	36	47
ABG-7022 + Dipel 2X	86	94	98	61	93	100	41	56	65	14	40	46
ABG-7022 + Dipel 2X Emul	72	91	98	72	96	98	28	54	70	0	11	16
Control	0	1	2	0	2	5	0	0	1	0	0	1

1/ Days of outside exposure
2/ Days larvae exposed to plants

Table 41. Percent defoliation of oak seedlings treated with a number of *Bt* formulations at 12 BIU/gallon/acre and exposed to 2nd instar gypsy moth larvae.

	Percent defoliation							
	Day 0[1/]		Day 2		Day 7		Day 8	
Material	2 day[2/]	4 day	2 day	4 day	2 day	4 day	2 day	4 day
E2492	22	68	14	21	20	56	17	54
E2492 UV	23	60	19	30	17	36	11	40
Dipel 8L	27	58	22	29	19	60	17	42
Foray 48B	15	46	4	9	9	32	28	47
ABG-7022 + Dipel 2X	1	5	3	5	4	12	6	20
ABG-7022 + Dipel 2X Emul	2	8	2	8	5	10	8	32
Control	52	80	50	90	26	50	16	42

1/ Days of outside exposure
2/ Days larvae exposed to plants

On September 1, 1989, single red oak trees (6-8 feet) were treated with Foray, Dipel 8L and E2492 using a backpack mist blower. Each tree was treated to the point of run-off with solutions of 16 BIU/gallon. Approximately 4 hours after treatment 500 laboratory reared gypsy moth larvae were introduced onto each tree. One untreated tree was used as a control. Test insects were 2nd and 3rd instar and had been reared on artificial diet.

Following a 24 hour exposure 100 larvae were removed from each tree and placed on artificial diet, 10 per dish. Test insects were then held in an environmental chamber at 78°F with 55% RH.

Mortality readings were made over a period of time.

Table 42. Percent mortality of gypsy moth larvae collected from oak trees treated with three formulations of *Bt*.

	Percent mortality after	
Material	2 days	4 days
E2492	74	95
Foray 48B	74	97
Dipel 8L	89	100
Control	1	1

We attempted to collect additional larvae off treated trees two days after treatment but were unable to find any.

A small laboratory test was conducted with 5 *Bt* formulations at 16 BIU/gallon/acre.

Table 43. Percent larval mortality and seedling defoliation following gypsy moth exposure to seedlings treated with 5 *Bt* formulations.

Material	Percent mortality			Percent defoliation		
	4 day	6 day	12 day	2 day	4 day	6 day
E-2492	51	66	98	8	20	27
E-2492 UV	53	69	100	6	13	23
Foray	69	93	100	3	7	12
ABG-7022 + Dipel 2X	86	98	100	1	3	3
ABG-7022 + Dipel 2X Emul	69	95	100	2	3	3
Control	0	0	1	86[1/]		

1/ changed to artificial diet

Laboratory tests were conducted with Biobit and Foray, *Bt* formulations from Novo Laboratories, Inc.

Table 44. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with 4 *Bt* formulations at 20 BIU/ gallon/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality		Percent defoliation	
		2 days	4 days	2 days	4 days
Foray	--	65	99	3	3
Foray	.25	39	83	10	25
Foray	.50	9	78	21	31
Foray	2.00	7	48	26	32
Biobit	--	51	96	5	7
Biobit	.25	50	83	3	3
Biobit	.50	44	98	7	12
Biobit	2.00	20	87	9	13
Dipel 8L	--	50	90	5	7
Dipel 8L	.25	1	33	18	40
Dipel 8L	.50	3	16	28	66
Dipel 8L	2.00	0	16	30	62
Dipel 8AF	--	39	98	5	6
Dipel 8AF	.25	9	57	6	19
Dipel 8AF	.50	7	56	27	38
Dipel 8AF	2.00	1	18	28	52
Control	--	0	0	54	94
Control	2.00	0	0	80	100

Table 45. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Foray *(Bt)* at various dosages.

Dosage/rate	Percent mortality				Percent defoliation			
BIU/gal/acre	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
16	2	35	56	70	2	4	7	12
24	12	65	93	99	1	2	2	2
30	15	80	93	100	1	1	1	1
35	16	63	93	100	1	1	2	2
40	16	61	87	100	2	2	2	22
48	13	67	93	99	1	2	2	2
Control	0	0	0	0	53	90	96	100

Table 46. Percent mortality of 3rd and 4th instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with 4 *Bt* formulations at 20 BIU/gallon/acre.

		Percent mortality			Percent defoliation		
Material	Instar	2 days	3 days	4 days	2 days	3 days	4 days
Foray	III	62	82	96	2	2	2
	IV	44	73	91	4	8	8
Biobit	III	38	75	94	2	2	2
	IV	29	62	90	2	2	2
Dipel 8L	III	23	33	71	5	8	8
	IV	23	41	71	17	17	32
Dipel 8AF	III	42	71	93	2	4	4
	IV	47	58	83	11	14	24
Control	III	0	0	0	100		
	IV	0	0	0	100		

Table 47. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to seedlings treated with Foray 48B and NuFilm 17 at 16 BIU/gallon/acre and then exposed to rainfall.

Amount of sticker	Inches rain	Percent mortality			Percent defoliation		
		2 days	4 days	5 days	2 days	4 days	5 days
No sticker	--	64	88	94	3	3	3
No sticker	.5	19	38	54	5	26	30
No sticker	1.0	4	14	44	14	34	44
No sticker	2.0	9	22	52	9	24	50
.5% NuFilm 17	--	40	76	89	2	3	6
.5% NuFilm 17	.5	25	40	63	6	20	24
.5% NuFilm 17	1.0	7	12	35	5	22	30
.5% NuFilm 17	2.0	28	36	61	7	23	29
1.0% NuFilm 17	--	50	54	82	3	13	13
1.0% NuFilm 17	.5	38	46	73	7	21	24
1.0% NuFilm 17	1.0	10	29	61	8	22	24
1.0% NuFilm 17	2.0	3	6	45	8	36	42
2.0% NuFilm 17	--	34	70	90	3	11	11
2.0% NuFilm 17	.5	30	39	53	7	26	28
2.0% NuFilm 17	1.0	15	27	45	9	24	31
Control	--	0	0	1	94	100	
Control	2.0	0	0	1	84	100	

A simulated ground application (Mistblower) was applied to oak seedlings using one quart of Foray 48B in 100 gallons of water and applied to seedlings at the equivalent of one gallon per acre. No mortality had occurred after 4 days and seedling defoliation averaged 80 percent.

Table 48. Percent larval mortality and oak seedling defoliation following a 4 and 5 day exposure to oak seedlings treated with 16 BIU/gallon/acre of Foray 48B and Complex 500 and then exposed to rainfall.

Percent Complex 500	Inches rain	Percent mortality		Percent defoliation	
		4 days	5 days	4 days	5 days
--	--	95	100	3	3
--	1.0	48	63	16	35
1.0	--	100		1	
1.0	1.0	60	75	20	38
5.0	--	78	92	5	6
5.0	1.0	54	70	25	32
10.0	--	95	99	3	3
10.0	1.0	50	66	33	41
Control	--	1	1	85	88
Control	1.0	0	0	99	100

Table 49. Percent larval mortality and oak seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with 2 samples of Foray 48B at 16 BIU/gallon/acre and then exposed to rainfall.

Foray 48B sample	Inches rain	Percent mortality		Percent defoliation	
		2 days	4 days	2 days	4 days
I[1/]	--	63	92	3	4
I	.25	37	54	11	37
I	.5	26	47	9	33
I	1.0	25	56	9	33
I	2.0	12	33	22	36
I	3.0	10	25	33	45
II[2/]	--	50	91	4	10
II	.25	36	61	5	17
II	.5	39	62	7	23
II	1.0	22	50	14	31
II	2.0	34	63	11	25
II	3.0	33	65	19	40
Control	--	0	0	90	100
Control	3.0	0	0	92	100

1/ Original sample sent to this laboratory (1 quart).
2/ second sample sent to this laboratory (2.5 gallons).

Table 50. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with Foray 48B-1 at 16 BIU/gallon/acre and various stickers with exposure to rainfall.

Sticker 2% by volume	Inches rain	Percent mortality 2 days	Percent mortality 4 days	Percent defoliation 2 days	Percent defoliation 4 days
—	--	66	97	3	4
—	1.0	30	77	12	21
NuFilm 17	1.0	48	85	5	12
Chevron	1.0	9	41	16	34
Complex 500	1.0	20	52	18	29
Plyac	1.0	30	75	10	21
Bond	1.0	44	90	3	7
Control	--	0	0	96	100
Control	1.0	0	0	93	100

Bond sticker *should not* be used with diluted applications of Foray 48B regardless of laboratory test results (Table 51). We recently discovered in Mission, Texas, that Bond is not compatible with diluted Foray in water. the mix clogged 50-mesh screens and caused the formulation to separate. When first mixed, the material looks fine, but starts to separate about 30 minutes after mixing.

Additional laboratory work was conducted with Foray 48B and Bond following the Mission, Texas trials. It was again confirmed in the laboratory that Bond *is not* compatible with water and Foray. However, when Bond was mixed at 2 percent by volume with Foray only, there appeared to be no problem with separation.

Other stickers such as Plyac, Chevron, NuFilm 17 and Complex 500 were mixed with Foray and water and found to be compatible with *Bt* (see Table 50 for efficacy results).

Table 51. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with 5 *Bt* formulations at 16 BIU/gallon/acre.

Formulation	Percent mortality 2 days	Percent mortality 4 days	Percent defoliation 2 days	Percent defoliation 4 days
Foray 48B (reg. - Texas)	50	98	1	1
Foray 48B (2705 - Texas)	59	99	2	2
Foray 48B (4008 - Texas)	68	93	1	2
Biobit 32B (Texas)	66	94	2	2
Dipel 8L (reg. - Texas)	16	31	16	35
Control	0	0	72	96

Table 52. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with undiluted Foray 48B* and exposed to rainfall.

Dosage/rate/acre	Inches rain	Percent mortality 2 days	Percent mortality 4 days	Percent defoliation 2 days	Percent defoliation 4 days
12 BIU/32 oz.	--	81	98	3	3
12 BIU/32 oz.	.5	34	81	11	20
12 BIU/32 oz.	1.0	13	43	23	45
16 BIU/43 oz.	--	77	99	4	4
16 BIU/43 oz.	.5	22	53	19	41
16 BIU/43 oz.	1.0	19	68	15	25
20 BIU/53 oz.	--	85	100	2	2
20 BIU/53 oz.	.5	22	66	14	25
20 BIU/53 oz.	1.0	14	61	17	25
40 BIU/106 oz.	--	99	100	1	1
40 BIU/106 oz.	.5	22	51	16	21
40 BIU/106 oz.	1.0	24	61	10	22
Control	--	0	0	85	92
Control	1.0	0	1	92	98

*Foray 48B was from that used in Mission, Texas, January 23, 1989 - February 2, 1989.

Table 53. Percent larval mortality and seedling defoliation following a 4 and 5 day exposure to oak seedlings treated with 7 *Bt* formulations at 16 BIU/gallon/acre.

Formulation	Percent mortality 4 days	Percent mortality 5 days	Percent defoliation 4 days	Percent defoliation 5 days
Foray 48B (Texas)	95	99	3	3
Thuricide 32LV	79	97	3	3
SAN-415 (NRD-12)	84	97	3	3
Dipel 8L	69	90	12	15
Dipel 8AF	95	97	2	2
Condor OF (PA)	94	99	4	4
Condor OF (TX)	84	97	7	8
Control	1	2	92	100

Table 54. Percent mortality of II, III and IV instar gypsy moth larvae and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with undiluted Foray 48B (Texas) at various dosages.

	II instar				III instar				IV instar			
	% mortality		% defoliation		% mortality		% defoliation		% mortality		% defoliation	
BIU/oz/acre	2 days	4 days	2 days	4 days	2 days	4 days	2 days	4 days	2 days	4 days	2 days	4 days
16/43 oz.	78	100	2	2	55	100	4	4	12	88	20	25
20/53 oz.	85	100	1	1	75	100	2	2	31	90	18	25
25/67 oz.	89	100	2	2	74	100	1	2	21	92	18	25
30/80 oz.	89	99	1	1	78	99	2	3	39	96	5	5
Control	0	0	94	100	0	0	100	100	0	0	100	100

Table 55. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with Foray 48B (Texas) at 16 BIU/gallon/acre with NuFilm 17 and exposed to rainfall.

Percent NuFilm 17	Inches rain	Percent mortality		Percent defoliation	
		2 days	4 days	2 days	4 days
--	--	48	92	4	4
--	2.0	0	3	24	72
0.5	--	66	99	3	3
0.5	2.0	0	1	33	70
1.0	--	53	97	2	3
1.0	2.0	0	3	22	58
2.0	--	35	95	3	4
2.0	2.0	1	2	30	60
Control	--	0	0	78	94
Control	2.0	0	0	78	100

Tender red oak seedlings were treated with 6 *Bt* formulations at 16 BIU/gallon/acre. Following a 2-hour drying time, 20 newly moulted 2nd instar gypsy moth larvae were exposed to each plant for a period of 5 minutes. Larvae were then removed to artificial diet and reared in an environmental chamber at 78°F with 50% RH.

Mortality readings were made over a period of time.

Table 56. Percent larval mortality following a 5-minute exposure to oak seedlings treated with *Bt* and then reared on artificial diet.

Formulation	Percent mortality				
	2 days	4 days	6 days	12 days	16 days
Dipel 8AF	0	8	11	19	28
Dipel 8L	1	2	4	6	9
Foray 48B (Texas)	1	2	4	6	10
Thuricide 32LV	1	2	3	4	6
SAN-415 (NRD-12)	1	2	3	8	8
Condor OF	1	2	4	5	7
Control	0	0	1	2	3

A feed study was conducted with 8 *Bt* formulations. The main objective was to determine what *Bt* formulation is preferred when 2nd instar gypsy moth larvae fed on *Bt*-treated oak foliage. A final analysis was made by determining percent of feeding on treated foliage over a period of time.

Tender oak seedlings were treated in a laboratory spray chamber with each of 8 *Bt* formulations at 16 BIU/gallon/acre.

After the spray had dried on the foliage for 2 hours, 1.0 inch diameter foliage discs were cut out of each treated seedling. Treated discs were then placed in a plastic container on top of moist filter paper. Each container contained 1 disc of each treatment and discs were evenly separated throughout. Forty newly moulted 2nd instar gypsy moth larvae were then placed into each container and given their choice as to what disc they wanted to feed upon. The tests contained 10 replications with each having treated discs located in different locations within each container. Each container had one untreated control. Percent defoliation of each disc was recorded after 18, 26 and 48 hours. All replications were then averaged to establish one figure for each treatment time.

Table 57. Average defoliation of 1.0 inch *Bt* treated oak foliage discs following exposure to 2nd instar gypsy moth larvae.

Formulation	Average defoliation of 10 replications		
	18 hours	26 hours	48 hours
Dipel 8L	9	13	16
Dipel 8AF	8	12	15
Thuricide 48LV	6	10	10
Thuricide 32LV	8	11	14
SAN-415 (NRD-12)	2	2	3
Foray 48B (Texas)	13	25	33
Condor OF	8	11	17
Condor AF	6	9	9
Control	5	10	13

A weathering test was conducted with 2 Condor formulations from Ecogen, Inc.

Table 58. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following exposure to seedlings treated with various *Bt* formulations and exposed to 0.5 inches of rainfall.

Material	Inches rain	Percent mortality				Percent defoliation			
		2 day	4 day	6 day	8 day	2 day	4 day	6 day	8 day
Condor 179-23	--	18	41	79	98	16	35	37	38
Condor 179-23	0.5	0	1	3	11	60	74	80	--
Condor 179-24	--	26	49	78	96	14	33	37	37
Condor 179-24	0.5	2	2	4	5	56	68	74	--
Foray 48B	--	56	92	98	100	2	3	3	3
Foray 48B	0.5	4	12	63	92	26	58	68	72
SAN-415 (NRD-12)	--	10	32	96	100	6	13	13	13
SAN-415 (NRD-12)	0.5	0	5	56	75	24	50	56	56
Dipel 8L	--	9	17	40	72	25	44	52	56
Dipel 8L	0.5	0	0	1	7	72	78	--	--
Dipel 8AF	--	26	56	86	99	7	16	16	16
Dipel 8AF	0.5	0	5	26	56	30	50	54	--
Control	--	0	0	0	1	60	--	--	--
Control	0.5	0	0	0	1	68	--	--	--

The registered formulations of *Bt* were tested for wash-off using various amounts of rainfall. Drying time was 3 hours before being exposed to rainfall.

Table 59. Percent larval mortality and oak seedling defoliation following a 4 and 5 day exposure of gypsy moth to oak seedlings treated with 7 *Bt* formulations at 16 BIU/acre undiluted and exposed to rainfall.

Formulation	Inches rain	Percent mortality after 4 days	Percent mortality after 5 days	Percent defoliation after 4 days	Percent defoliation after 5 days
Dipel 8L	--	64	83	15	15
Dipel 8L	0.1	4	19	74	74
Dipel 8AF	--	81	93	8	8
Dipel 8AF	0.1	11	31	66	70
Condor OF	--	65	75	14	20
Condor OF	0.1	1	3	94	98
Condor AF	--	35	64	44	48
Condor AF	0.1	27	44	58	64
Foray	--	83	93	8	8
Foray	0.1	38	75	24	26
Thuricide 48LV	--	73	97	9	9
Thuricide 48LV	0.1	38	53	43	47
Thuricide 32LV	--	37	69	22	26
Thuricide 32LV	0.1	25	48	7	13
Control	--	0	0	100	100

Table 60. Percent larval mortality and oak seedling defoliation following a 4 and 6 day exposure of gypsy moth to oak seedlings treated with 7 *Bt* formulations and exposed to rainfall.

Formulation	Inches rain	Percent mortality after 4 days	Percent mortality after 5 days	Percent defoliation after 4 days	Percent defoliation after 5 days
Dipel 8L	--	56	89	19	23
Dipel 8L	.25	5	21	85	92
Dipel 8AF	--	73	97	10	13
Dipel 8AF	.25	7	65	41	46
Condor OF	--	40	83	46	53
Condor OF	.25	0	25	93	93
Condor AF	--	4	40	68	74
Condor AF	.25	4	41	82	86
Foray	--	83	99	11	11
Foray	.25	14	67	69	70
Thuricide 48LV	--	70	90	11	14
Thuricide 48LV	.25	2	59	78	80
Thuricide 32LV	--	39	80	18	18
Thuricide 32LV	.25	12	24	35	56
Control	--	0	2	100	100

Table 61. Percent larval mortality and oak seedling defoliation following a 4-day exposure of gypsy moth to oak seedlings treated with 7 *Bt* formulations at 16 BIU/acre and exposed to rainfall.

Formulation	Inches rain	Percent mortality after 4 days	Percent defoliation after 4 days
Dipel 8L	--	72	13
Dipel 8L	.5	2	84
Dipel 8AF	--	71	18
Dipel 8AF	.5	19	40
Condor OF	--	76	16
Condor OF	.5	3	68
Condor AF	--	13	50
Condor AF	.5	33	56
Foray	--	86	10
Foray	.5	39	44
Thuricide 48LV	--	34	24
Thuricide 48LV	.5	12	67
Thuricide 32LV	--	50	21
Thuricide 32LV	.5	12	15
Control	--	0	100

Table 62. Percent larval mortality and oak seedling defoliation following a 4 and 5 day exposure of gypsy moth to oak seedlings treated with 7 *Bt* formulations at 16 BIU/acre undiluted with 1% Bond sticker and exposed to rainfall.

Formulation	Inches rain	Percent mortality after 4 days	Percent mortality after 5 days	Percent defoliation after 4 days	Percent defoliation after 5 days
Dipel 8L	--	67	85	9	9
Dipel 8L	.1	10	40	56	60
Dipel 8AF	--	76	88	8	8
Dipel 8AF	.1	38	62	50	50
Condor OF	--	55	68	40	40
Condor OF	.1	17	29	74	74
Condor AF	--	28	52	76	76
Condor AF	.1	35	53	42	44
Foray	--	77	93	19	19
Foray	.1	35	56	41	43
Thuricide 48LV	--	52	67	19	20
Thuricide 48LV	.1	29	57	52	52
Thuricide 32LV	--	45	81	18	18
Thuricide 32LV	.1	24	47	20	32
Control	--	0	0	100	100

A number of *Bt* field samples were tested for Forest Service.

Table 63. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following exposure to red oak seedlings treated with a number of *Bacillus thuringiensis* formulations at 16 BIU/gallon/acre.

Formulation	Percent mortality			Percent defoliation		
	4 days	6 days	8 days	4 days	6 days	8 days
Dipel 8AF 27-123	67	100		11	11	
Dipel 8AF 27-126	50	98	100	22	22	
Dipel 8AF 27-123 27-126	63	99	100	17	17	
Dipel 8L 28-298	11	45	70	54	77	82
Dipel 8L 28-305	18	67	82	38	59	85
Foray 48B 6016	70	97	99	6	7	7
Foray 48B 6018	66	94	99	13	13	15
Foray 48B (Lab Std.)	74	97	100	6	6	
Dipel 8L (Lab Std.)	5	52	65	58	77	
Dipel 8AF (Lab Std.)	56	94	98	15	22	
Control	0	0	0	66	100	

Table 64. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following exposure to red oak seedlings treated with a number of *Bacillus thuringiensis* formulations at 8 BIU/gallon/acre.

Formulation	Percent mortality			Percent defoliation		
	4 days	6 days	8 days	4 days	6 days	8 days
Dipel 8AF 27-123	7	77	92	10	32	42
Dipel 8AF 27-126	28	72	91	13	13	13
Dipel 8AF 27-123 27-126	18	70	91	21	30	36
Dipel 8L 28-298	2	3	11	18	64	
Dipel 8L 28-305	4	5	13	22	64	
Foray 48B 6016	47	94	100	15	17	17
Foray 48B 6018	78	99	100	4	5	5
Foray 48B (Lab Std.)	61	96	99	8	12	12
Dipel 8L (Lab Std.)	1	7	11	74		
Dipel 8AF (Lab Std.)	7	71	85	52	54	
Control	0	0	0	88		

Table 65. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following exposure to red oak seedlings treated with a number of *Bacillus thuringiensis* formulations.

Material	Dosage BIU/gal/acre	Percent mortality		Percent defoliation	
		4 days	5 days	4 days	5 days
Dipel 8L (1) 16-639 BJ	4	1			
"	8	4			
"	12	6			
"	16	21	39	50	65
"	20	42	54	29	41
Dipel 8L (2) 16-653 BJ	4	0			
"	8	10			
"	12	10			
"	16	8	16	54	74
"	20	41	58	16	28
Dipel 8L (3) 16-652 BJ	4	5			
"	8	2			
"	12	14			
"	16	9	20	38	49
"	20	48	73	10	15
Dipel 8L (4) 16-639 BJ	4	4			
"	8	2			
"	12	14			
"	16	7	16	52	69
"	20	41	51	13	28
Dipel 8L (5) 16-652 BJ	4	2			
"	8	3			
"	12	5			
"	16	30	41	42	54
"	20	51	62	24	19
Dipel 8L (6) 16-653 BJ	4	0			
"	8	4			
"	12	7			
"	16	31	57	34	43
"	20	30	36	35	44
Dipel 8L Lab Std.	4	0			
"	8	4			
"	12	3			
"	16	8	27	56	71
"	20	34	44	16	25
Dipel 8L Lab Std.	4	4			
"	8	35			
"	12	64			
"	16	93	96	3	3
"	20	95	100	3	3
Control	--	1	2	94	100

A number of feeding tests were conducted with the feeding stimulant Entice.

First we conducted two feeding tests with 1" treated oak leaf discs comparing the feeding of second instar gypsy moth larvae on untreated (control) versus treated foliage. Two 1" oak leaf discs cut form oak foliage (one treated, one untreated) were placed in a plastic petri dish lined with damp filter paper. Five early second instar larvae were introduced onto the dish and allowed to feed for a certain amount of time. Each group was replicated five times.

The foliage was treated using two different methods -- the Brush and the Spray Tower method. The Spray Tower methods involved treating the whole oak seedling in the spray tower, simulating an aerial application of a gallon/acre. The Brush method involved brushing the gallon solution onto the whole surface of the leaf. After the foliage was dry, 1" leaf discs were cut from the leaves.

Tables 66 and 67 show the results.

Table 66. Average percentage of larval feeding after 18 hours on Entice-treated vs. untreated oak leaf discs -- Spray Tower method (five 2nd instar larvae per dish).

Treatments	100% Entice	75% Entice	50% Entice	25% Entice	10% Entice	Control
Treated	98	82	90	98	62	94
vs						
Control	54	83	85	52	90	97

Table 67. Average percentage of larval feeding on Entice-treated vs. untreated oak leaf discs after 20 hours -- Brush method (five 2nd instar larvae per dish).

Treatments	100% Entice	75% Entice	50% Entice	25% Entice	10% Entice	Control
Treated	84	99	96	89	79	66
vs						
Control	86	63	24	60	46	60

For the third test, the number of larvae per dish was decreased to prolong the period of time to observe the feeding preferences. Table 68 shows the results.

Table 68. Average percentage of larval feeding after various hours on Entice-treated vs. untreated oak leaf discs -- Brush method (two 2nd instar larvae per dish).

Treatments	100% Entice	25% Entice	20% Entice	15% Entice	10% Entice	7% Entice	5% Entice	3% Entice	1% Entice	100% H_20	Control
Feeding after 17 hours:											
Treated	4.6	25.0	17.0	15.0	34.0	32.5	28.4	39.0	31.0	21.0	16.2
vs											
Control (untreated)	4.2	4.0	11.2	11.0	7.4	12.0	11.4	10.2	18.2	12.2	18.6
Feeding after 24 hours:											
Treated	12.6	50.0	34.0	30.0	70.0	55.0	54.0	52.0	62.0	42.0	23.2
vs											
Control (untreated)	11.0	13.0	19.4	18.0	14.0	19.0	18.4	17.4	26.2	22.0	38.0
Feeding after 42 hours:											
Treated	34.2	83.0	77.0	68.2	90.0	100.0	88.0	86.0	78.0	82.0	46.0
vs											
Control (untreated)	24.0	44.2	28.0	34.0	43.0	65.0	54.0	56.0	67.0	60.0	62.0
Feeding after 48 hours:											
Treated	44.2	88.0	90.0	78.4	96.0	100.0	94.0	94.0	94.0	94.0	58.0
vs											
Control (untreated)	37.0	70.2	42.0	42.0	59.0	84.0	66.0	68.0	84.0	82.0	76.0

Since the previous tests definitely show a larval preference for Entice over the untreated foliage, we decided to next conduct a feeding comparison test between two mixes of Entice, seven *Bacillus thuringiensis* formulations, and a control.

In this comparison test, 1" oak leaf discs of each treatment were placed in a large rectangular plastic dish; thus, 10 discs per dish. The discs were in a random order. This design was replicated 10 times. Then early 2nd instar larvae were introduced into each dish. Table 69 shows the results of three tests -- two using the Spray Tower method, one using the Brush method.

Table 69. Feeding comparison test of 2 Entice mixes, 7 *Bt* formulations, and Control. Average percentage of feeding after 24 hours (all *Bt* formulations at 16 BIU/gal/solution).

Treatment	Test A Spray Tower Method	Test B Spray Tower Method	Test C Brush Method
Dipel 8L	8.7	5.3	.7
Biobit	6.2	9.0	.3
San-415	5.0	5.5	.7
Condor Oil	3.6	7.1	.2
Foray	5.6	11.0	1.7
Thuricide 48LV	1.4	7.5	.9
Entice 5%	29.5	46.5	10.0
Entice 15%	22.6	35.0	11.2
Dipel 8AQ	5.7	8.0	1.1
Control	14.1	31.0	9.2

Six tests were conducted with Dimilin during this period.

Testing was conducted using our standard red oak seedling technique. Five seedlings were treated with each dilution and then exposed to 20 newly moulted laboratory-reared gypsy moth larvae. Plants and larvae were then held in an environmental chamber at 78°F at 55% RH. Larval mortality was recorded over a period of time. When seedlings were completely defoliated, larvae were transferred to artificial diet.

Table 70. Percent mortality of 2nd instar larvae following a 7-day exposure to oak seedlings treated with undiluted applications of Dimilin (special low volume formulation) and exposed to rainfall.

Dosage/rate/acre	Inches rain	Larval mortality after a 7-day exposure
.03 lbs. AI/16 oz.	-	100
.03 lbs. AI/16 oz.	1.0	100
.02 lbs. AI/16 oz.	2.0	100
.03 lbs. AI/32 oz.	-	100
.03 lbs. AI/32 oz.	1.0	100
.03 lbs. AI/32 oz.	2.0	100
.015 lbs. AI/32 oz.	-	100
.015 lbs. AI/32 oz.	1.0	100
.015 lbs. AI/32 oz.	2.0	100
Control	-	0
Control	2.0	14

Table 71. Percent mortality of 2nd instar larvae following 4-7 and 10 day exposure to oak seedlings treated with undiluted applications of Dimilin (special low volume formulation) and exposed to rainfall.

Formulation	Inches rain	Hours drying time	Percent mortality 4 days	7 days	10 days
Dimilin B	-	-	39	100	100
.03 lbs. AI/32 oz./A	2.0	.25	46	95	100
	5.0	3.5	52	100	100
Dimilin C	-	-	41	100	100
.03 lbs. AI/16 oz./A	2.0	.25	35	100	100
	5.0	3.5	47	100	100
Dimilin E	-	-	49	100	100
.015 lbs. AI/32 oz./A	2.0	.25	68	99	100
	5.0	3.5	38	100	100
Control	-	-	0	0	0
	5.0	-	0	0	1

Table 72. Percent mortality of 2nd instar larvae following a 4 and 7 day exposure to oak seedlings treated with Dimilin 2F material used for 1987 field tests in West Virginia.

Sample	Dosage/rate	Percent mortality 4 days	8 days
I	.0625 lbs. AI/32 oz./A	70	100
	.0312 lbs. AI/16 oz./A	61	100
	.0156 lbs. AI/8 oz./A	76	100
	.0078 lbs. AI/4 oz./A	58	82
II	.0312 lbs. AI/32 oz./A	66	98
	.0156 lbs. AI/16 oz./A	59	100
III	.0625 lbs. AI/16 oz./A	63	100
	.0312 lbs. AI/8 oz./A	47	98
	.0156 lbs. AI/4 oz./A	63	90
Control	--	1	1

Table 73. Percent mortality of 2nd instar larvae following a 4 and 7 day exposure to oak seedlings treated with Dimilin (special formulations) used in 1988 Pennsylvania field trials.

Dosage/rate/acre	Percent mortality 4 days	7 days
.03 lbs. AI/16 oz.	84	100
	76[1/]	100
.03 lbs. AI/32 oz.	76	100
.03 lbs. AI/64 oz.	58	100
.03 lbs. AI/128 oz.	79[2/]	100
Control	0	2

1/ After material was strained
2/ Dimilin 25W

Small amounts of Dimilin 25W were applied to oak seedlings and then exposed to 2nd instar gypsy moth larvae. A droplet 400 ul in size was used for the test.

Table 74. Percent mortality of 2nd instar larvae following a 4-9 and 14 day exposure to oak foliage treated with single and multiple droplets of Dimilin 25W.

Mix	Droplets per	Percent mortality 4 days	9 days	14 days
.06 lbs./gal/sol	1 drop/leaf	15	40	50
	1 drop/cm^2	42	91	91
	3 drops/cm^2	29	100	100
.03 lbs./gal/sol	1 drop/leaf	28	58	80
	1 drop/cm^2	51	99	100
	3 drops/cm^2	46	98	100
Control	--	0	0	0

Table 75. Percent mortality of 2nd instar larvae following an 8-day exposure to oak seedlings treated with Dimilin on various degrees of wet foliage.

Formulation	Dosage/rate acre	Foliage degree of wetness	Percent mortality
Dimilin 25W	.03 lbs./gal	Dry	100
Dimilin 25W	.03 lbs./gal	wet to point of run-off[1/]	100
Dimilin B	.03 lbs./32 oz.	Dry	100
Dimilin B	.03 lbs./32 oz.	wet to point of run-off[1/]	100
Dimilin C	.03 lbs./16 oz.	Dry	100
Dimilin C	.03 lbs./16 oz.	wet to point of run-off[1/]	99
Dimilin E	.015 lbs./32 oz.	Dry	100
Dimilin E	.015 lbs./32 oz.	wet to point of run-off[1/]	100
Control	--	Dry	2

1/ Seedlings were dripping wet at time of Dimilin application.

Dimilin gave excellent results in all tests. Mortality was complete after a heavy 5.0 inches of rain.

San-839I and San-841I, insect growth regulators from Sandoz, Inc., were tested in the laboratory and compared to Dimilin 25W.

Table 76. Percent larval mortality and oak seedling defoliation following gypsy moth larvae exposure to seedlings treated with San-829I, San-841I and Dimilin 25W.

Material	Dosage/rate lbs.AI/gal/acre	Percent mortality 4 day	8 day	12 day	Percent defoliation 2 day
San-839I	.05	8	59	79	92*
"	.025	2	67	78	98*
"	.012	0	58	100*	
San-841I	.05	3	100		80*
"	.025	9	83	100[1/]	78*
"	.012	21	92	100[1/]	
Dimilin 25W	.05	1	86	100[1/]	94*
"	.025	4	96	100[1/]	94*
"	.012	5	75	100	100
Control	--	0	0	0	100

1/ Reading after 10 days
* Changed to artificial diet

A test was conducted in which each material was exposed to various amounts of rainfall with bioassay following. Plants were treated with the "Insect Growth Regulators" and allowed to dry for three hours before being exposed to rainfall. After rainfall plants were dried in front of a fan and then tested as in Table 77. All testing was done at .03 lbs. AI/gallon/acre.

Table 77. Percent larval mortality and oak seedling defoliation following gypsy moth larvae exposure to seedlings treated with San-839I, San-841I and Dimilin 25W and then exposed to rainfall.

Material	Inches rain	Percent mortality 2 day	6 day	10 day	Percent defoliation 2 day
San-839I	--	0	23	43	84
"	1.0	0	10	50	78
"	3.0	0	10	71	76
"	5.0	0	5	39	85
San-841I	--	0	100		87
"	1.0	0	99	100	100
"	3.0	0	99	100	47
"	5.0	0	100		70
Dimilin 25W	--	0	99	100	72
"	1.0	0	97	100	94
"	3.0	0	96	100	52
"	5.0	0	97	100	72
Control	--	0	0	0	83
"	5.0	0	0	0	80

* Larvae were changed to artificial diet after 2 days' exposure to treated foliage

In these tests San-841I was as effective as the standard Dimilin 25W in both dosage and weathering tests. San-839I was less effective than San-841I and Dimilin 25W. Heavy seedling defoliation occurred with all materials in the first two days of test insect exposure. This is common for "Insect Growth Regulators" using this test technique.

Standard weathering tests were conducted with EXP-60145A, an experimental material from Rhone-Poulenc Agricultural Co. Treated plants were dried for 3 hours before being exposed to rainfall.

Table 78. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following exposure to Rhone-Poulenc experimental material 60145A.

Pounds	Percent mortality					Percent defoliation				
Al/gal/acre	1 day	2 day	3 day	4 day	7 day	1 day	2 day	3 day	4 day	7 day
1.0	2	22	78	94	100	5	7	7	8	8
0.5	1	21	75	99	100	5	5	5	6	6
0.25	0	17	76	91	100	11	17	17	17	17
0.125	0	10	67	86	100	22	30	32	34	34
0.062	0	17	50	83	99	30	38	42	44	49
0.031	0	10	50	83	99	36	54	54	54	61
0.015	0	10	36	55	86	40	58	66		
0.007	0	3	13	29	47	42	68	78		
Control	0	0	0	0	0	46	84	88		

Table 79. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following exposure to Rhone-Poulenc experimental material 60145A with application of rainfall 3 hours after insecticide was applied.

Pounds	Rainfall	Percent mortality					Percent defoliation				
Al/gal/acre	1.0"	1 day	2 day	3 day	4 day	7 day	1 day	2 day	3 day	4 day	7 day
1.0	no	3	34	76	99	100	4	5	6	6	
1.0	yes	1	23	75	100		4	4	5	5	
0.25	no	0	17	57	97	100	20	20	20	22	22
0.25	yes	0	11	32	96	100	20	20	20	21	21
0.06	no	0	11	32	97	100	34	40	40	44	44
0.06	yes	0	1	28	92	100	36	50	58	66	66
0.015	no	0	0	9	44	55	42	66	82		
0.015	yes	0	0	8	40	49	44	74	86		
Control	no	0	0	0	1	1	31	62	76		
Control	yes	0	3	3	5	5	40	84	94		

Table 80. Percent larval mortality and seedling defoliation following exposure to oak seedlings treated with EXP-60145A at various dosages and exposed to rainfall.

Dosage/rate lbs.AI/gal/acre	Inches rain	Percent mortality				Percent defoliation			
		2 day	3 day	5 day	7 day	2 day	3 day	5 day	7 day
.25	--	35	77	94	100	6	7	8	8
.25	4.0	7	48	96	100	12	18	18	18
.25	5.0	20	62	91	99	9	17	17	17
.25	--[1/]	25	79	99	100	5	7	7	--
.25	2.0[1/]	19	60	96	100	7	11	11	11
.25	3.0[1/]	8	59	99	100	15	16	19	19
.125	--	5	36	94	98	17	27	27	28
.125	1.0	10	40	94	100	11	19	23	23
.125	2.0	11	32	94	100	16	24	32	32
.125	3.0	2	34	96	98	34	34	36	36
.0625	--	14	51	95	100	14	25	25	25
.0625	1.0	4	30	90	97	38	40	46	48
.0625	2.0	2	34	89	97	38	56	60	64
.0625	3.0	3	20	64	74	58	70	70	70
.0625	4.0	6	68	77	100	18	30	31	32
.0625	5.0	3	35	95	97	27	45	46	46
.0625	--[1/]	13	64	97	100	12	14	15	15
.0625	2.0[1/]	1	37	90	99	23	26	26	28
.0625	3.0[1/]	0	22	86	100	41	50	50	50
.0312	--	3	27	92	99	44	52	54	56
.0312	1.0	5	15	69	75	16	50	70	--
.0312	2.0	6	18	51	52	48	66	--	--
Control	--	0	0	0	0	50	78		
Control	5.0	0	1	1	1	74	100		

1/ Plants aged for 24 hours after treatment and before exposure to rainfall.

Project Number: GM 81.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leaders: John Allen Tanner, Susan E. Lane and Heidi M. Thatcher

The purpose of this project is to monitor the development of the laboratory strains and to determine if it falls within the acceptable ranges. Data are collected for each strain/generation and are used to detect changes from normal development so that corrective action can be taken.

The Otis New Jersey standard strain (ONJSS) has been greatly influenced by the larval "straggling" syndrome and other developmental problems over the last several generations (Tanner and Weeks, 1980; Tanner et al, 1984; Tanner et al, 1986, Tanner et al, 1988). We are now collectively calling these performance problems Abnormal Performance Syndrome or APS.

The ONJSS had a severe problem with APS during the 1988-89 rearing year (G33 and G34). Figure 1 shows that the percentage of egg masses producing "straggling" larvae (mean larval stage 1.00 - 1.25; Tanner et al, 1984) rose to its highest level (23%) in G33 and is averaging 17% thus far in G34. The percentage of core samples which produced no mean larval stage either due to non-hatch of the eggs or death of all the neonates before they were 11 days old also reached its highest level in G33 with only a slight drop in the percentage thus far in G34 (Figure 2).

Figure 3A shows that the average hatch of a random sample of 20 egg masses varied considerably between production weeks. It ranged from a high of 90% to a low of 20%. Figure 3B shows a similar variation in the percentage of egg masses classified as having APS (n=100 masses). As expected there was a high negative correlation coefficient (-.748 Pearson) between the percentages. The higher the percent hatch the lower the number of egg masses classified as having APS.

In the last report (Tanner et al, 1988) we showed some disturbing trends in the development of the ONJSS. We observed general decreases in larval survival, the average weight of female pupa and adult female emergence. These data indicate that the ONJSS has deteriorated over the last several generations.

Inbreeding may be one possible cause for this deterioration. Animals used to maintain the colony are normally reared weekly and this has resulted in the development of several subcolonies that have been genetically isolated from each other for several generations.

During 1988-89, we attempted to reduce inbreeding by doing outcrossing between "adjacent" subcolonies. Figure 4 shows the method used for each week of production. Two pure matings (male and female from the same subcolony) and two cross matings (male and female from different subcolonies) were generally made each week.

Outcrossing was started midway through G33. The performance of the insects used during the crossmating period was generally better than that observed in the insects used at the beginning of G33 but similar to those used in the G32 (Table 1).

Outcrossing had no influence on the reproductive performance of the ONJSS (Table 2); i.e., the pure and outcrossed lines had similar results. The reproductive performance in G34 was better than that observed in G33 but similar to that observed in G32.

The first 5 weeks of G33 cross matings have been evaluated through the offspring generation (G34). The effect of outcrossing on the incidence of APS can not be determined at this time. Neither the pure line nor the outcrossed line had a high incidence of APS even though both parent subcolonies used each week did have a high percentage of egg masses classified as having APS (Figure 5). This indicates that APS is probably not a genetic trait but more of a nutritional-environmental problem or possibly due to an interaction between the two.

Outcrossing had no influence on the percent embryonation or hatch in the offspring (Figure 6), nor did it increase the percentage of offspring larvae that survived to the pupal stage (Figure 7). Offspring male and female pupal deformity (Figure 8) and male and female adult emergence and deformity (Figure 9) were similar in the pure and cross lines.

Percent hatch of F_1 eggs produced during the 1988 sterile insect program (G32 and G33) was the lowest of all the years that this program has been conducted (Figure 10). These eggs also produced a high percentage of "straggling" larvae and therefore were not used for field release the following spring (Mastro, personal communication).

References Cited

Tanner, J. A., J. J. Baker, H. A. Hamilton and B. A. Fontes. 1986. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1985 - September 30, 1986:122-125.

Tanner, J. A., J. J. Baker, M. Matthews, Jr., N. Garvey and S. E. Lane. 1988. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1987 - September 30, 1988:272-276.

Tanner, J. A., J. J. Baker and M. Palmeri. 1984. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1983 - September 30, 1984:77-79.

Tanner, J. A., and B. P. Weeks. 1980. Establishing Standards. APHIS Laboratory Report, October 1,1979 - September 30, 1980:167-175.

Table 1. Performance of New Jersey Colony insects.

Parameter	Generation 1/ 32 (no cross) 2/ mean	SE	n	33 (no cross) mean	SE	n	33 (cross) mean	SE	n	34 (cross) mean	SE	n
Larvae per cup	7.9	0.05	35	10.2	0.54	25	9.6	0.18	23	11.1	0.19	48
Mean larval stage 3/	2.87	0.04	35	2.90	0.09	23	2.93	0.05	23	2.95	0.04	48
Pupal weights (gms)												
female	2.01	0.04	35	1.99	0.04	25	2.06	0.04	23	1.95	0.03	48
male	--	--	--	0.67	0.01	25	0.67	0.01	23	0.66	0.01	48
% Survival neonate to pupae	82.5	1.22	35	66.2	4.07	25	75.6	2.74	23	79.9	1.49	48
Developmental time to 50% pupation (DT50)												
female	27.4	0.21	35	29.4	0.50	25	27.1	0.18	23	27.5	0.12	48
male	25.2	0.15	35	27.4	0.52	25	25.5	0.21	23	26.2	0.14	48
Sex ratio M:F	1.1:1	0.03	35	1.3:1	0.10	25	1.2:1	0.15	23	1.1:1	0.03	48
% Adult emergence												
female	89.7	2.93	35	88.8	2.70	25	95.3	1.23	23	98.2	0.31	48
male	--	--	--	96.0	1.23	25	98.3	0.39	23	97.7	0.49	48
% Adult deformity												
female	8.9	1.53	35	19.4	3.24	25	14.0	1.14	23	10.3	1.25	48
male	--	--	--	5.6	1.36	25	1.7	0.68	23	0.9	0.17	48

1/ Generation 32 - started January 7, 1988 through September 9, 1988.
Generation 33 - started September 17, 1988 through May 22, 1989.
Generation 34 - started May 22, 1989 through September 30, 1989.

2/ No cross = one subcolony started per week.
Cross = two subcolonies started per week. Pure and cross matings were made for evaluation in the next generation.

3/ 9th Post-neonate infest date.

Table 2. Reproductive data of New Jersey colony insects.

Parameter	Generation 1/ 32 (no cross) 2/ mean	SE	n	33 (no cross) mean	SE	n	33 (pure) mean	SE	n	33 (cross) mean	SE	n	34 (pure) mean	SE	n	34 (cross) mean	SE	n
% of females depositing an egg mass	81.6	2.6	35	70.6	4.6	25	78.1	5.7	23	76.7	4.3	23	84.4	2.1	43	83.4	1.8	44
# eggs deposited per female	834.5	36.4	35	700.8	47.2	25	685.1	50.9	23	753.2	43.2	23	777.9	25.7	43	730.5	20.7	44
# colony eggs per colony mating	634.7	29.4	35	538.3	43.1	25	593.8	43.1	23	591.6	32.1	23	647.8	14.2	43	638.7	12.9	44

1/ Generation 32 - started January 7, 1988 through September 9, 1988.
Generation 33 - started September 17, 1988 through May 22, 1989.
Generation 34 - started May 22, 1989 through September 20, 1989.

2/ No cross = one subcolony started per week.
Pure = two subcolonies started per week with matings made between adults from the same subcolony.
Cross = two subcolonies started per week with matings made between adults from the opposite subcolony.

Figure 1. Percentage of Otis New Jersey colony egg masses that produced "straggling" larvae.

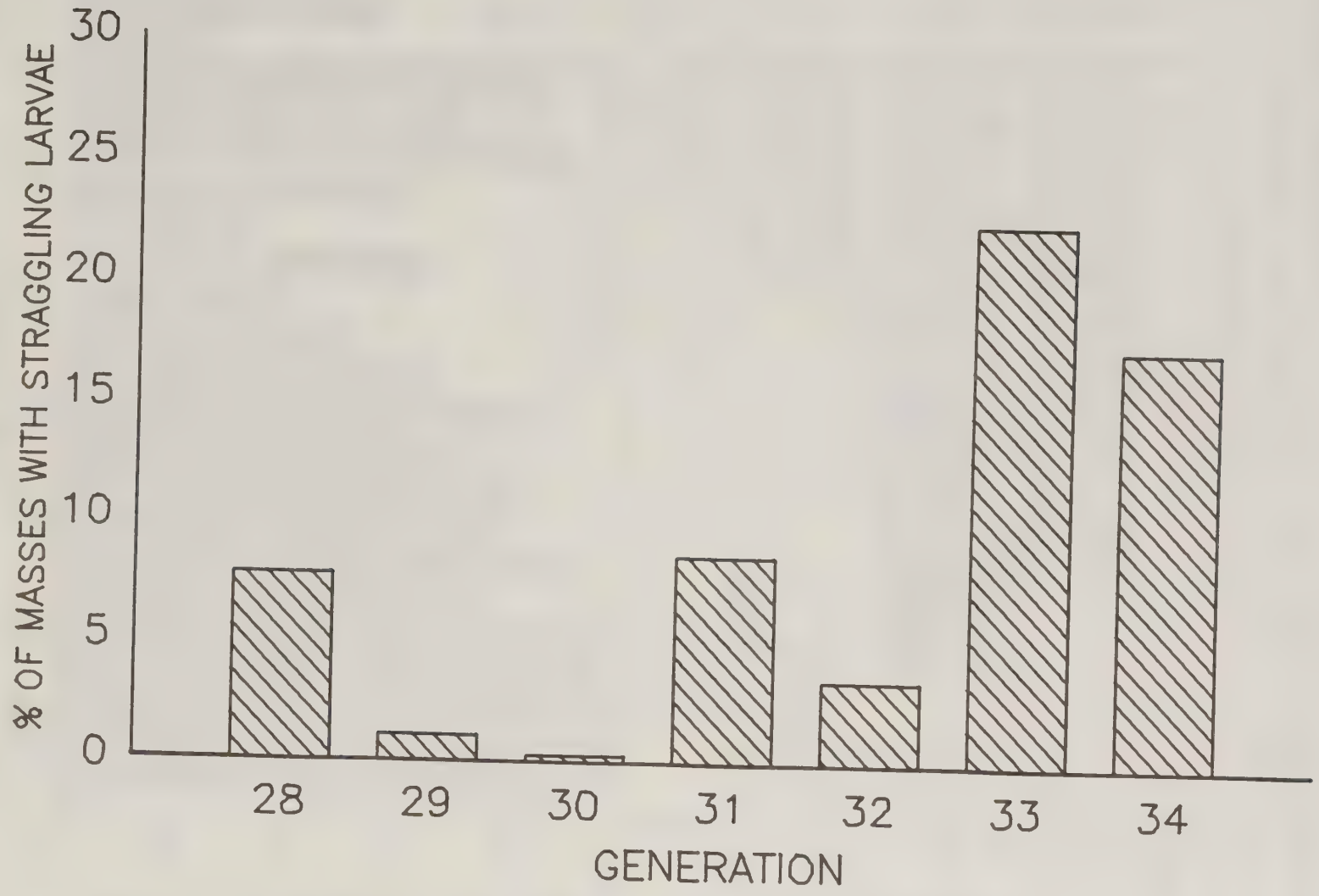


Figure 2. The % of New Jersey colony core samples that produced no mean larval stage due to non hatch of the eggs or death of all the neonates.

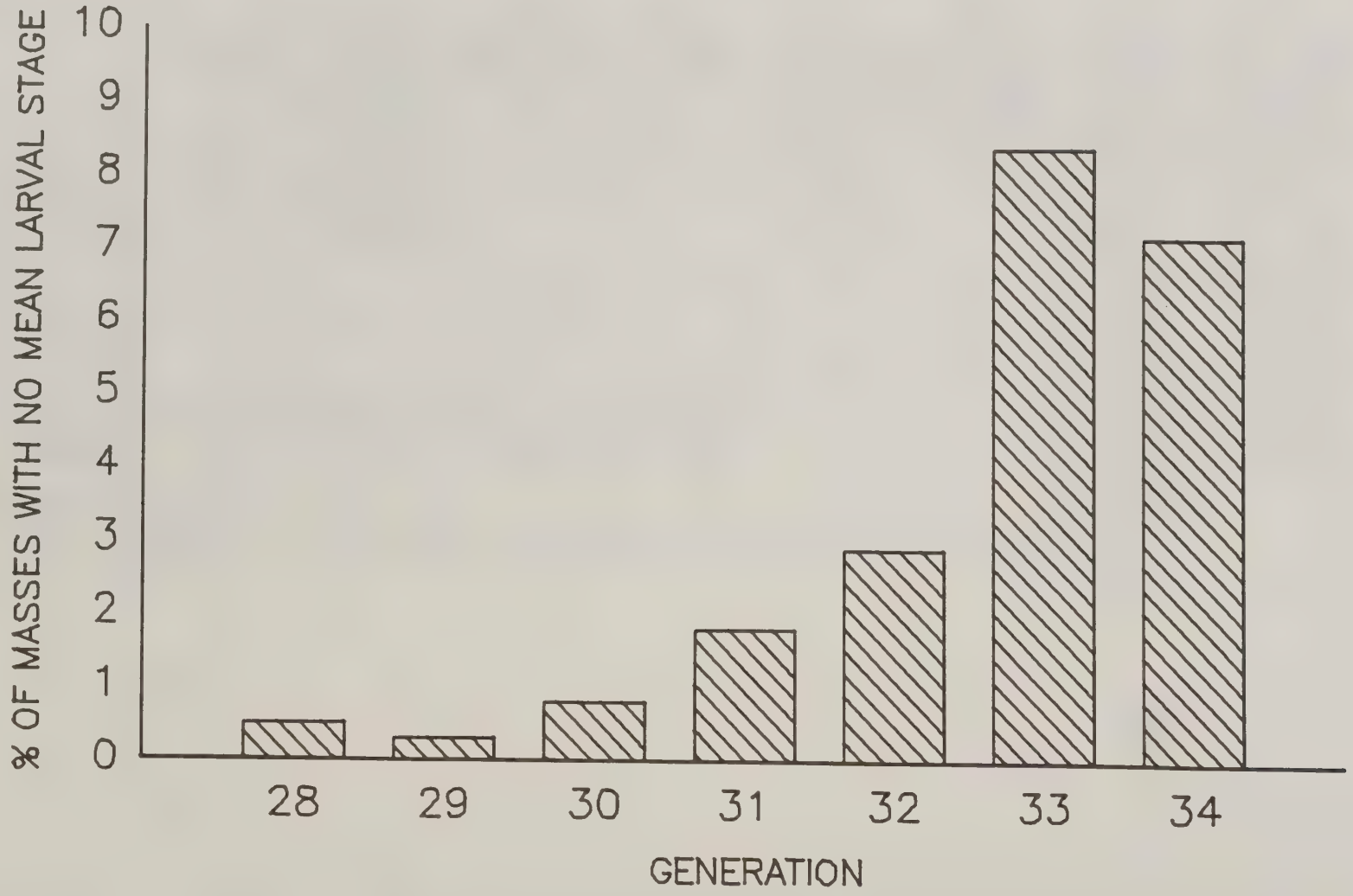


Figure 3. Mean percent hatch (A) and percentage of egg masses classified with APS (B) in the Otis New Jersey strain. (Pearson's corr. = −.749)
vertical bars indicate ±1 S.E.

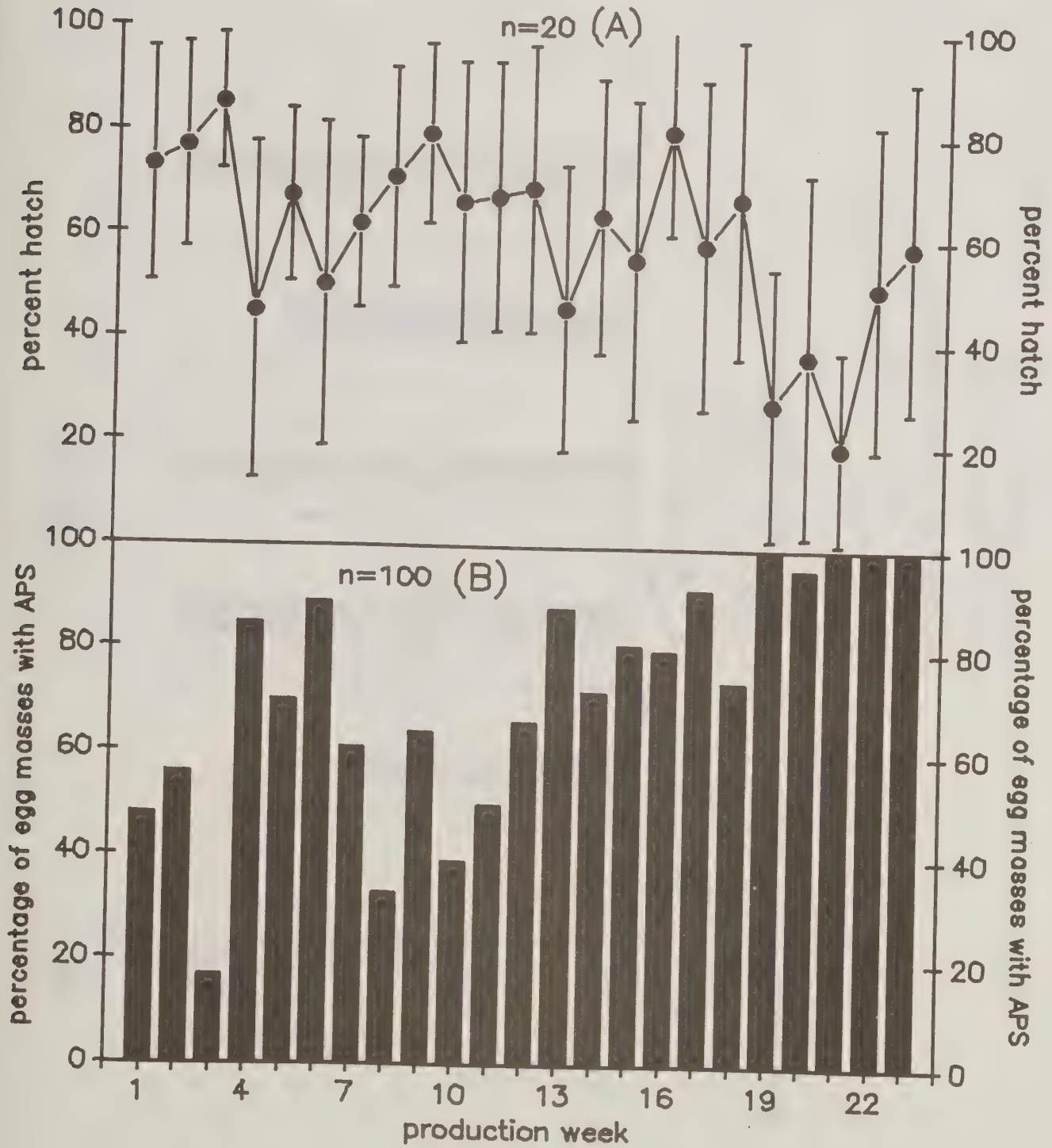


Figure 4. Mating scheme used during the 1988-89 rearing year.

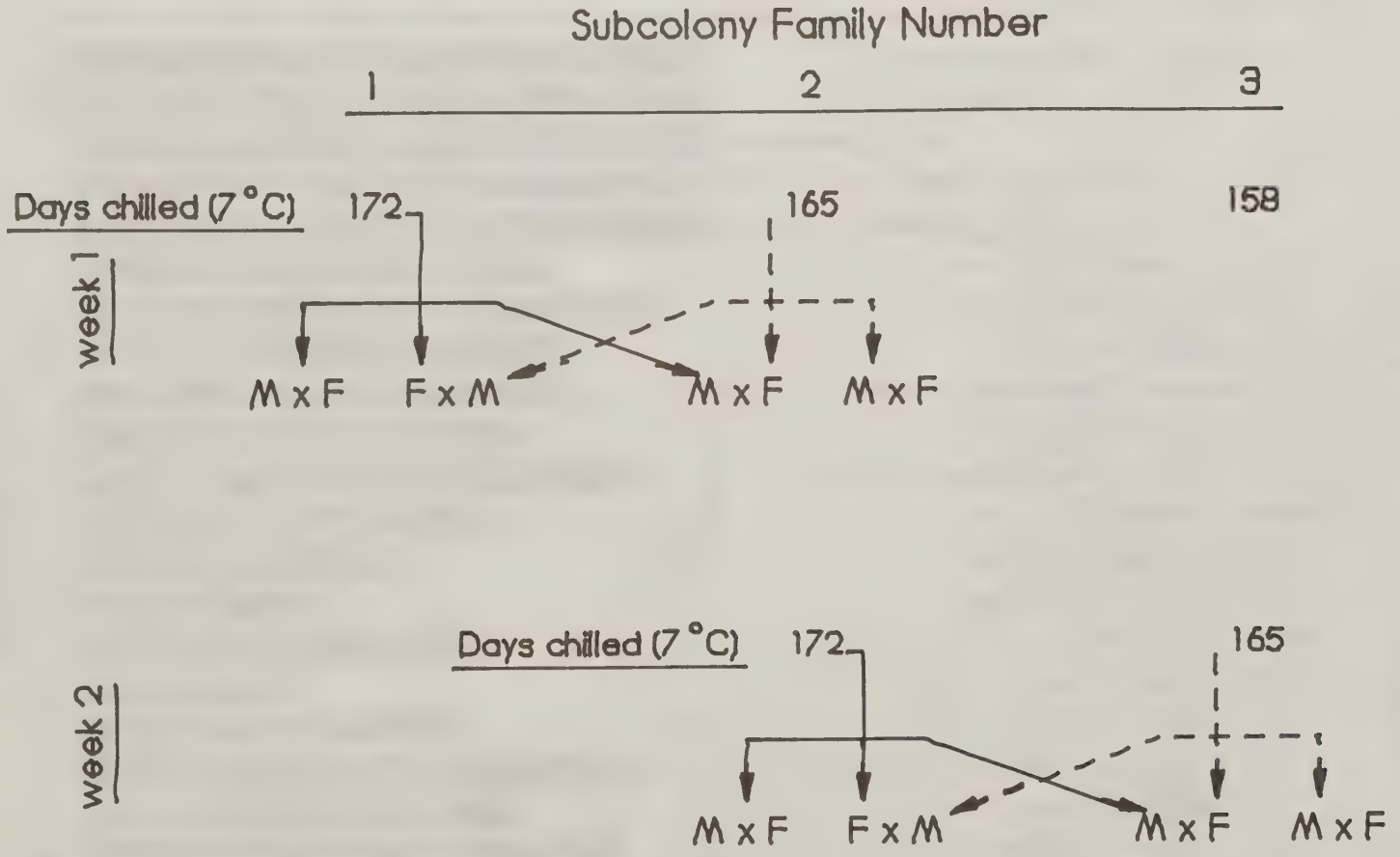


Figure 5. Percentage of abnormal performance syndrome (APS) egg masses in the Otis New Jersey strain subcolonies used during the first five weeks of crossmatings.

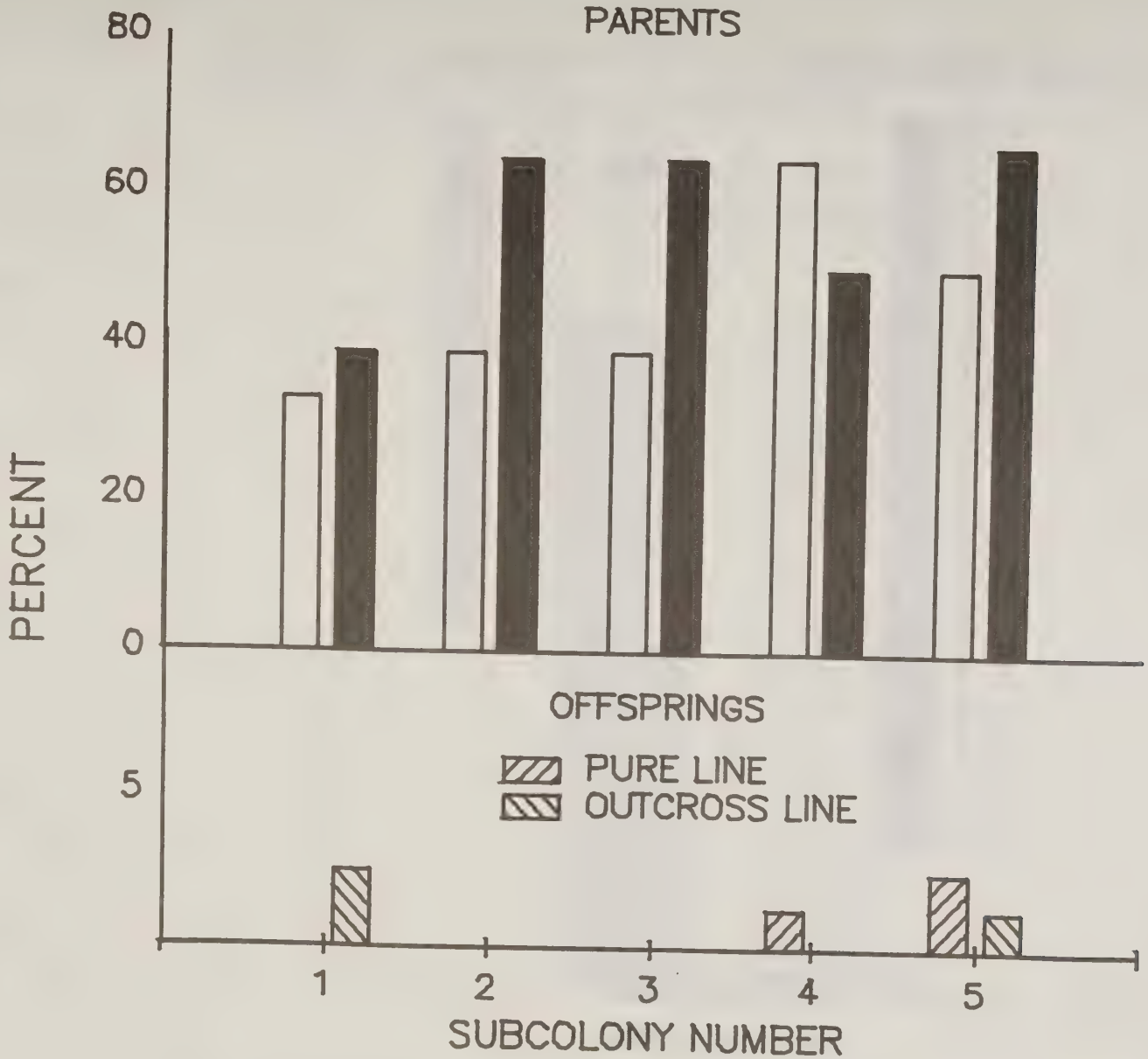


Figure 6. Percent hatch and embryonation of Otis New Jersey colony eggs from the first five weeks of colony crossmatings.

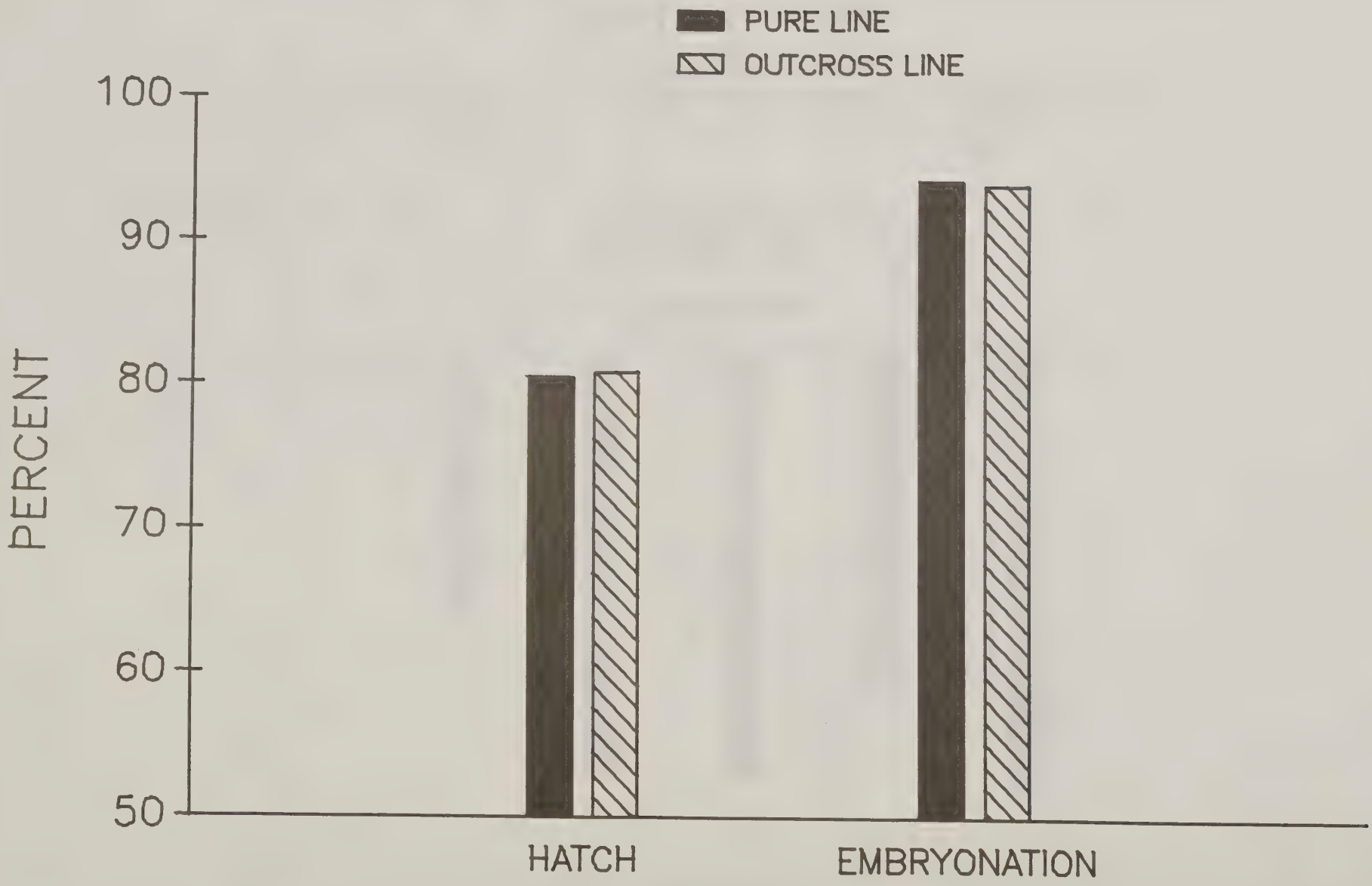


Figure 7. Percent larval survivial to the pupal stage in the first five weeks of Otis New Jersey colony crossmatings.

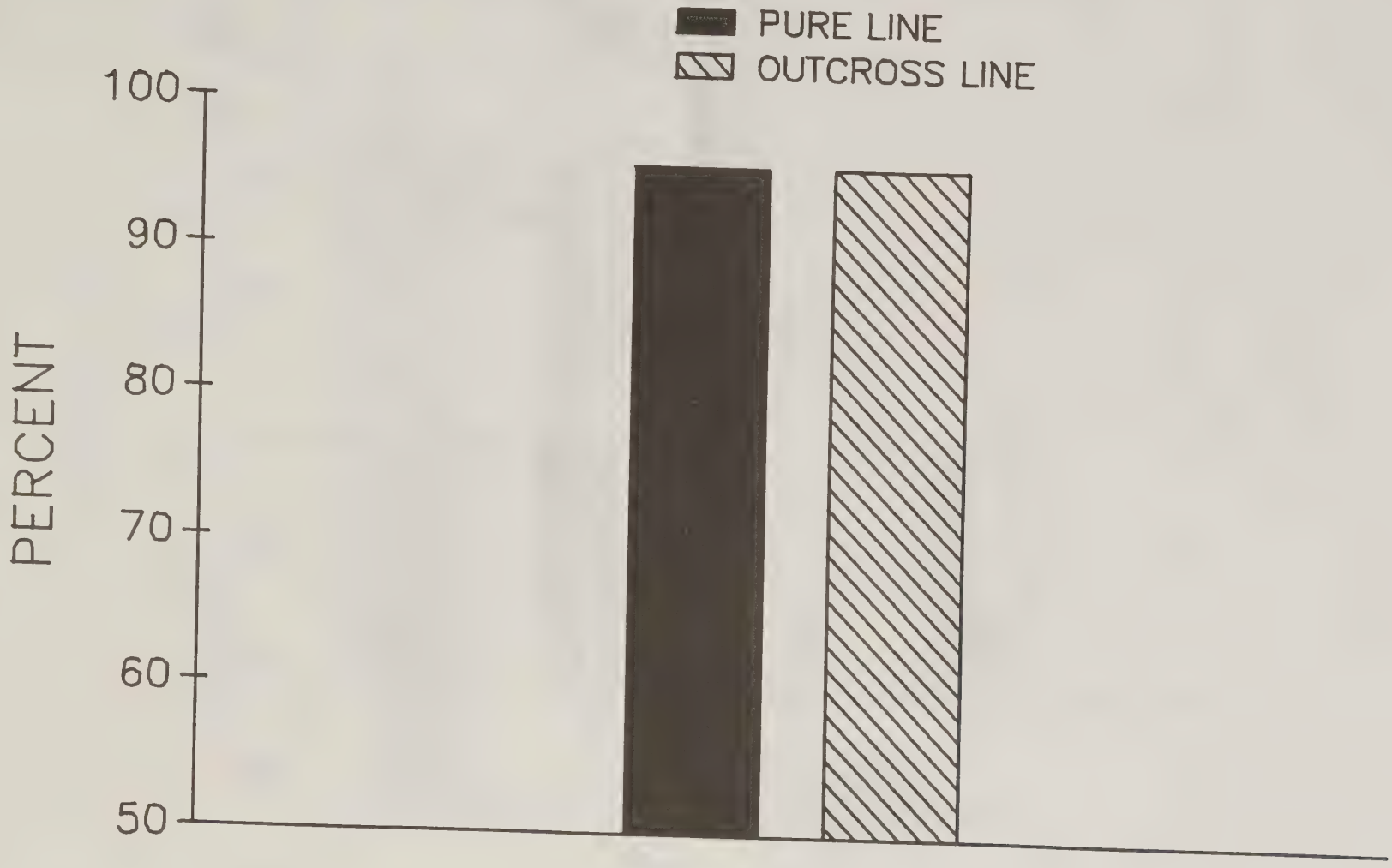
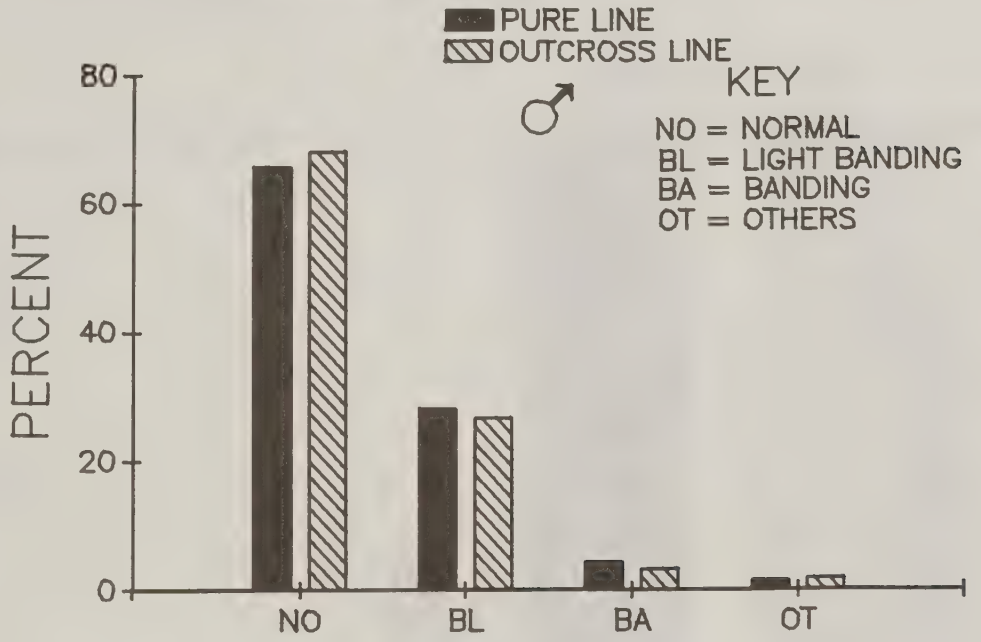


Figure 8. Percent male and female pupal deformity in the first five weeks of Otis New Jersey colony crossmatings.



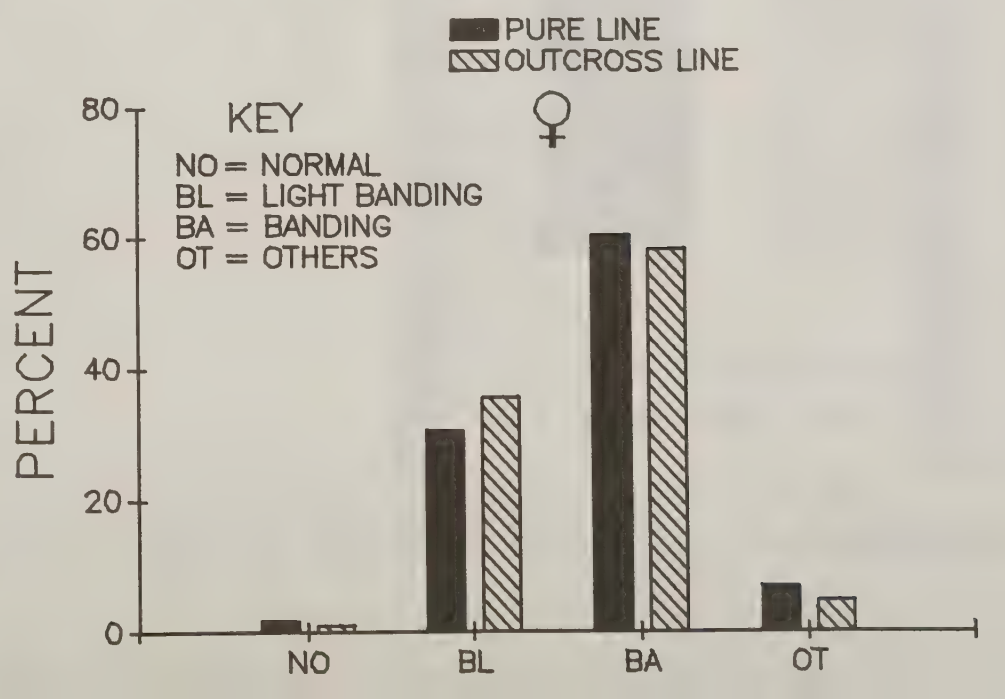


Figure 9. Percent male and female adult emergence and deformity in the first five weeks of Otis New Jersey colony crossmatings.

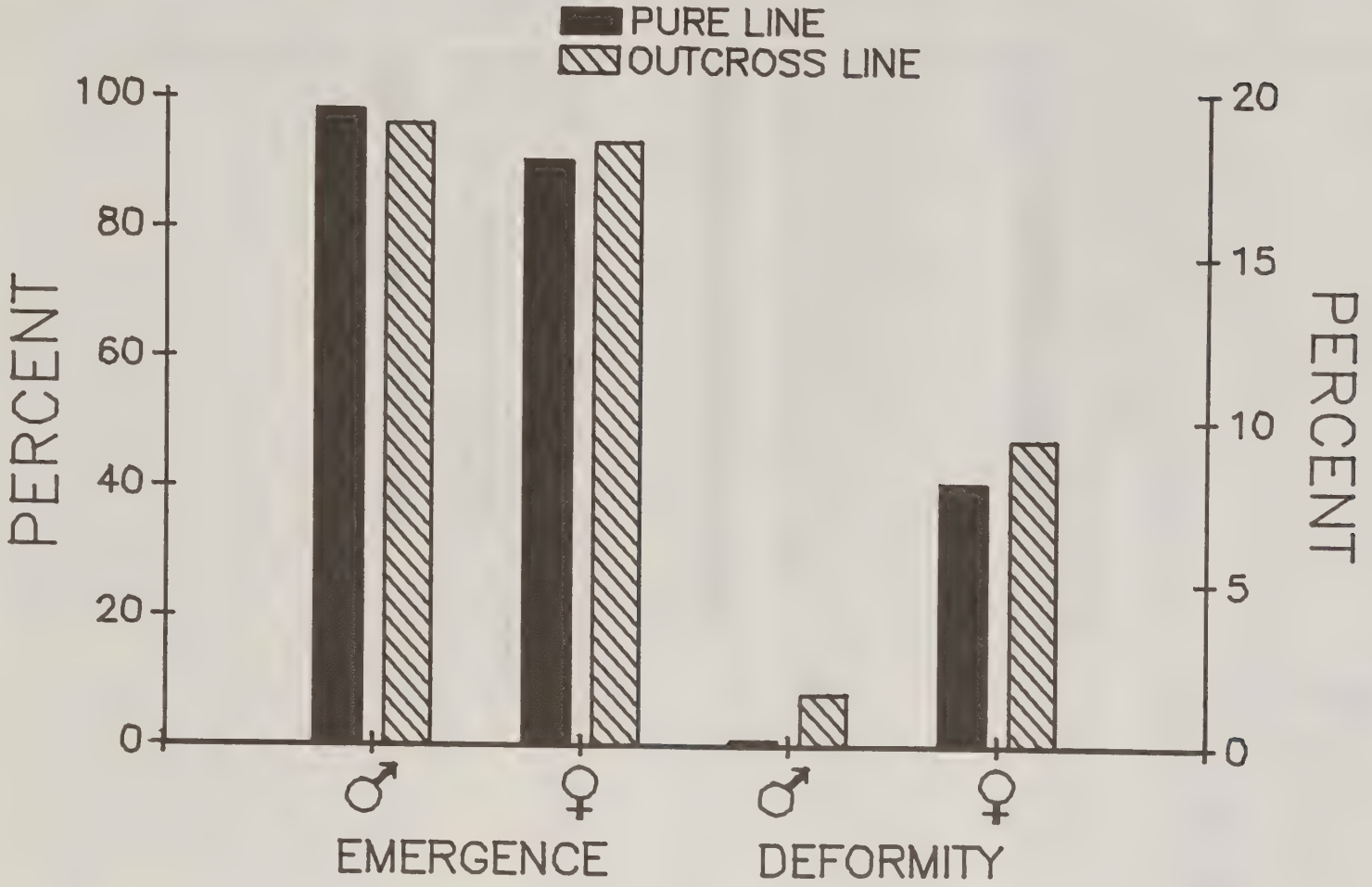
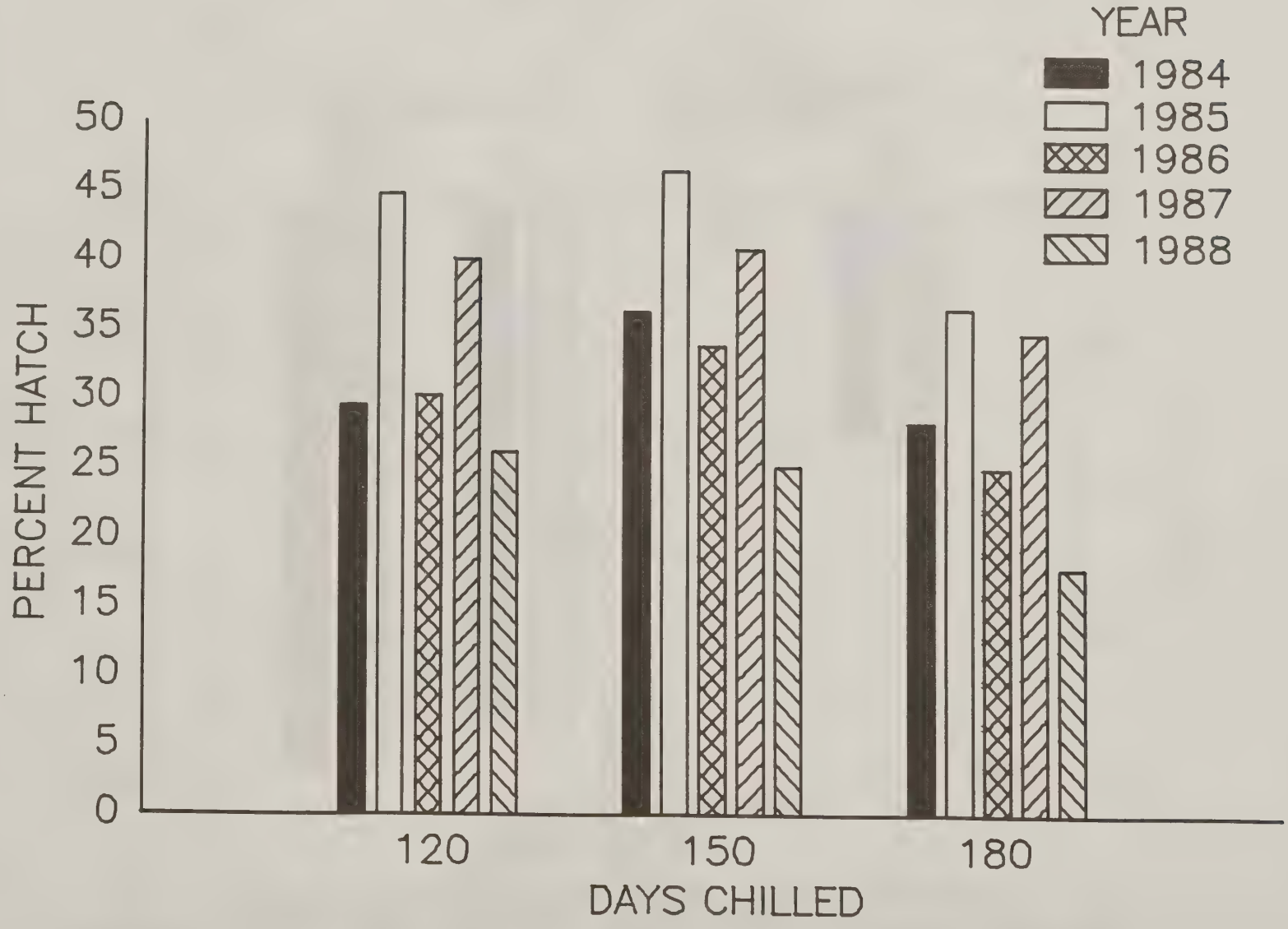


Figure 10. Percent hatch of F1 partially sterilized eggs



Project Number:	GM 83.3.1
Project Title:	Development and Evaluation of Improved Rearing Techniques
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leaders:	John Allen Tanner, J. A. Finney, O. T. Forrester, Susan E. Lane and Heidi M. Thatcher

This project concerns the development and evaluation of new rearing techniques and the improvement of techniques presently in use. Unreliable or inefficient techniques will be modified or discarded.

SAMPLING METHOD USED IN THE F_1 STERILE MALE PROGRAM

In the F_1 sterile male program, male pupae should be irradiated when they are 6 to 9 days old (4-day window). Irradiating male pupae younger than 6 days or older than 9 days usually results in reduced egg hatch in F_1 generation (Mastro and Schwalbe, 1988). The date for irradiating the male pupae is determined from a pupal age curve (PAC) and is done when the maximum or peak number of male pupae are within the critical 6-9 day age window.

Estimating the true PAC is difficult. Rearing carts (2/day) are placed front to back in the environmental rooms. This allows little room for maneuvering the carts for sampling. Only those cups located along the end rows of each cart are accessible. Sampling is done by parting the two carts then selecting two cups from the top and bottom of each cart by random numbers (2 cups/location/cart; 8 cups/day). New cups are selected each day and the total number of male pupae counted. Counts are made from the 24 to 35 post egg infest date (PEID). The pupae are harvested on the 35th PEID.

The PAC is estimated by dividing the highest observed daily male pupal count into the increased number of male pupae counted on each day. Those days which had no increase or even a decrease were given a 0%. The four consecutive days that gave the highest total percent pupation are used to determine the irradiation date.

It had never been determined if this sampling method was reliable in predicting the population peak four days of male pupation or if there was a less time-consuming method. We attempted to do this in the following experiment.

Methods

This experiment was conducted during the F_1 sterile male program. Adults emergence curves were constructed instead of pupation curves in order to minimize disruption to the ongoing program. It was assumed that the adult emergence curve mimics the pupation curve. Two carts were used in each replication. The carts were placed in the normal front to back manner. The front cart was nearest the center rearing room aisle and the back cart nearest the wall. Every cup along the outer edges of a cart that faced the opposite cart was sampled (normal area for selecting sample cups) as well as every other cup(483 cups/cart; 966 cups/replication) on the rest of the cart. Sampling began on the 35th PEID after the pupae were removed from the non-selected cups and used in the F_1 sterile male program. These cups were returned to the same location on the carts after the pupae were harvested to help hold the sample cups in their previous location and maintain similar conditions as during pupation. Each sample cup was checked daily and adults removed and tallied by sex. The cups were checked until all emergence stopped. The sampling procedure described above was tested 20 times/replication and the number of correct and incorrect peak four-day predictions determined. Three replications were conducted with 2 weeks between each replication.

Results

In all three replications, the four consecutive days with the highest male emergence (peak four days) was similar for larvae reared on the end rows of each rearing cart compared to the population (Table 1). In replication 3, the front cart emergence peak deviated one day from the population emergence peak. However, this deviation was actually the result of delayed male emergence on the lowest level (7 levels/cart) of the cart (level 7). On the rest of the cart the peak four days of emergence coincided with that of the population.

The percentage of total males that emerged during the population peak four days was 62.4 ± 1.6 (Table 2). Assuming that the male pupation curve mimics the adult emergence curve, this would also be the maximum percentage of male pupae that could be in the critical 4-day irradiation window (Mastro and Schwalbe, 1988). Only 16 (26.7%) of the 60 samples correctly predicted the population peak four days (Table 2). Sample prediction was off ± 1 day in 24 (40.0%) of the samples and ± 2 or more days in 20 (33.3%) of samples.

If the sample prediction was off ± 1 day, the maximum percentage of male pupae in the correct age group (6-9 day old) would only have been 55.8-58.2% or a 4.2-6.6% reduction (Table 2). A ± 2-day divergence would have reduced the percentage to 39.7-48.7% or a 13.7-22.7 reduction. The above sampling method was off ± 2 or more days in 33.3% of the samples. This indicates that the above sampling methods was not very reliable. Increasing the sample size would increase the accuracy. However, this method is already time-consuming, often taking 3 or more hours/day at peak production. The most time-consuming part is the selection (by random numbers) of the sample cups on the carts (not the actual reading of the cups).

We tested the possibility of continuously reading the same sample cups (selected from end rows). With this system, the sample cups would only have to be selected (random number) on the initial day of reading. The cups could be marked for easier sighting on the following days. Once the cups are read, they would be returned to their exact location on the carts for the following day's reading.

This sampling method was tested for accuracy in predicting the population peak four days of emergence using the above raw test data.

Selecting 8 sample cups (2/location/cart) results in 18 (30.0%) of the 60 samples predicting the correct population peak four days (Table 2). Sample prediction was off ± 1 day in 31 (51.7%) of the samples and ± 2 or more days in 11 (18.3%) of the samples. This was only slightly better than the old sampling method.

This sampling method would undoubtedly take less time than the old method and therefore we attempted to increase the precision by doubling the sample size to 16 cups. Doubling the sample size increased the number of correct predictions to 25 (41.7%). Sample predictions were off ± 1 day in 30 (50.0%) of the samples and ± 2 or more days in 5 (8.3%) of the samples. Tripling the sample size to 24 cups increased the number of correct predictions to 39 (65.0%). Sample predictions were off ±1 day in 18 (30.0%) of the samples and ±2 or more days in 3 (5.0%) of the samples.

Conclusion

1. A population male emergence profile can be estimated using cups located on the last row of each rearing cart.

2. The method currently used to estimate the emergence profile is not reliable and is too time-consuming to allow for an increase in the sample size.

3. Continuous reading of the same sample cups is slightly more accurate than the original sampling method but less time-consuming (personal observations).

4. Increasing the continuous reading sample size to 12 cups/cart improved to 95% the accuracy in predicting within ±1 day the peak four days of male emergence **(Time factor involved in tripling the sample size must still be evaluated.)**

EFFECTS OF AGAR ON THE GROWTH OF OTIS NEW JERSEY STRAIN

Agar (Gracillaria) is used as the gelling agent in the Otis B-4 diet. The agar has been primarily of Japanese origin; however, in 1987 several agar shipments were of Moroccan origin. The Moroccan agar was finer and darker in color than the Japanese agar. Otis gypsy moth larvae reared on the original shipment of Moroccan agar developed similarly to those reared on the Japanese agar (Tanner unpublished data) and it was accepted for use in rearing. A later shipment of Moroccan agar was found to be much darker in color than the original shipment and at this time abnormal performance syndrome (APS) (Tanner et al, 1989) began to reappear in the colony and production insects.

In 1988 and 1989 we compared the development and reproduction of Otis gypsy moth larvae reared on the darker Moroccan agar against those reared on the original lighter Moroccan agar and on an agar of Japanese origin (Gracillaria). We also compared the darker Moroccan agar to a similar agar that had gone through centrifugation (washed) to remove the dark particles and a similar agar that had the washed supernatant added to it. Lastly, we compared a normal 2% concentration of Japanese agar to a 1% concentration.

Methods

Formalin treated (dehaired) eggs were hand-placed (approx. 20 eggs/cup) into each diet cup (11 cups/ treatment/replication) approximately 45 minutes after the diet was poured. The cups were held in a standard rearing room (25-26°C, 65-75% R.H.). The cups were opened after 7 days of incubation and counts made as to the number of hatched, unhatched, and unembryonated eggs and the number of dead larvae. The cups were opened a second time (7 days later) and the number of larvae in each instar and the number of dead larvae counted. Pupation curves were started on the 21st post egg infest date (PEID) and the pupae harvested, weighed and checked for deformity on the 35th PEID. Ten matings were made for each agar treatment. The resulting egg masses were weighed 35 days after mating. The eggs were then chilled (7°F) for 170 days. Two core plugs were taken out of each mass after they completed chilling. One plug was placed in a high humidity (80%+) chamber and hatch determined. The second plug was placed on B-4 diet and used to determine the incidence of abnormal performance syndrome (APS). The number of larvae/instar and the number of dead larvae and unhatched samples were determined 14 days later. Three replications were conducted at three week intervals.

Results

Table 3 shows that there were no consistent trends in the developmental differences between insects reared on the Japanese (2%) and the light and dark Moroccan agars (2%). Survival to pupation was significantly reduced when larvae were reared on either the light or dark Moroccan agar. Also, larvae reared on Moroccan agar had a higher percentage of deformed female pupae. All other developmental traits had only minor, insignificant differences. Days to 50% (DT50) male pupation was similar in all three agars, but female DT50's were one day later when larvae were reared on dark Moroccan agar.

Table 4 presents the data for the second generation. The agar used in the parent generation had no significant influence on egg mass weight, egg embryonation and hatch in the offspring. However, all the hatch rates were lower than expected. Highest hatch occurred when light Moroccan agar was used and lowest hatch with the dark Moroccan agar. Embryonation was highest with the Japanese agar and lowest with the dark Moroccan agar.

Abnormal performance syndrome (APS) (egg masses with a MLS [mean larval stage] <1.25; no hatch; or 100% mortality of neonates) was prevalent in all the treatments. However, all the non-hatched egg masses occurred in the first replication. The masses with a MLS of 1.25 or lower were equally distributed through all the replications. Parents reared on the light Moroccan agar produced the highest percentage of normal egg masses (43.3) but also produced the lowest percentage of egg masses (70.0%) (Table 4). The percentage of egg masses classified as having APS ranged from 56.7% (light Moroccan agar) to 78.5% (Japanese agar).

Removing the supernatant from the dark Moroccan agar (washed) seemed to increase pupal survival when compared to non-washed dark Moroccan diet (Table 3); however, when the supernatant was added to non-washed diet there was no reduction in survival. This indicates that the pupal survival results may be due to chance and not a treatment effect. Removing the supernatant had no effect on the G1 generation (Table 4). All treatment had a high degree of APS.

Halving the amount of Japanese agar in the B-4 diet significantly increased the percentage of deformed female pupae; however, this was the only significant effect seen in either generation (Tables 3 and 4). Reducing the amount of agar in the B-4 diet may be one way to reduce the cost of rearing gypsy moth and should be evaluated thoroughly.

Conclusion

1. Moroccan agar was not the APS-causing factor in the diet.

WHEAT GERM MEAL AS A SUBSTITUTE FOR WHEAT GERM

Difficulty at times in obtaining sufficient quantities of raw wheat germ (27% crude protein) has resulted in the production facility substituting wheat germ meal (25% crude protein) for wheat germ during the periods of scarcity.

In 1989 the development of Otis gypsy moth larvae on diet containing wheat germ meal was compared to similar larvae reared on diet containing wheat germ.

Methods

Newly eclosed neonates (8/cup) were placed on fresh diet containing either wheat germ or wheat germ meal. The cups (30 cups/treatment) were held in a standard rearing room (25-26°C, 65-75% R.H.). Each cup was opened after 7 days of incubation and counts were made as to the number of dead larvae and the number of larvae/instar. Each cup was opened a second time (7 days later) and the number of larvae in each instar and the number of dead larvae were counted. Pupation curves were started on the 21st post-neonate infest date (PNID) and the pupae harvested, weighed and checked for deformity on the 35th PNID. Ten matings were made for each treatment. The resulting egg masses were weighed 35 days after mating. The eggs were then chilled (7°F) for 170 days. Two core plugs were taken out of each mass after they completed chilling. One plug was placed in a high humidity (80%+) chamber and hatch determined. The second plug was placed on B-4 diet and used to determine the incidence of abnormal performance syndrome (APS). The number of larvae/instar and the number of dead larvae and unhatched samples were determined 14 days later. Three replications were conducted at weekly intervals.

Results

The only significant difference found between the two treatments was a higher percentage of female pupal deformity in the wheat germ meal treatment (Table 5). The higher pupal deformity had no impact on adult female emergence and their subsequent mating. APS was not a problem in either treatment (Table 6).

Conclusion

1. Wheat germ meal can be substituted for wheat germ in the B-4 diet without affecting the development of Otis gypsy moth larvae in the parent generation or influencing the hatch and incidence of APS in the G1 generation. **(The use of wheat germ meal over several generations still needs to be evaluated.)**

POTENTIAL MARKER FOR F_1 LARVAE

In the F_1 sterile male program, there is a need for a simple methods for field identification of F_1 larvae to monitor developmental synchrony, survival and overflooding ratios (Mastro and Schwalbe, 1988). The methods currently used are chromosome analysis and mating-egg mass evaluation. Both of these methods are time-consuming and the results are often obtained late in the season. A simpler method would be to have an external, genetically controlled anatomical marker that is not present in the wild population.

Many of the Otis gypsy moth larvae have a bright, white, shield-shaped mark (beauty mark) on the dorsal portion of the metathorax segments. An example of this can be seen in McManus and Twardus (1988). Each Otis gypsy moth subcolony has members with and without this mark. Last year we conducted mating tests to determine if this trait is genetically controlled.

Methods

Larvae were selected from colony production and segregated by the presence or absence of the mark when it first became visible after the first molt. All larvae were reared on B-4 diet until pupation.

The following four mating types were made using newly emerged adults: beauty ♂ x beauty ♀; beauty ♂ x non-beauty ♀; non-beauty ♂ x beauty ♀; non-beauty ♂ x non-beauty ♀. After a minimum of 150 days (7°C) of chilling, ten egg masses were selected weekly from each mating type and incubated. Fifty larvae were removed from each mass before the mark became apparent (after the first molt). The larvae from each mating type were reared in 6 oz. cups (10/cup) with 85 mls. of B-4 diet. The percentage of larvae with the mark was determined on the 7th, 14th and 21st post-neonate infest days to ascertain the persistence of the mark through the larval period.

Results

The data in Table 7 indicates that the beauty mark trait is genetically controlled and dominant. The lack of a 50:50 ratio in the cross matings indicates that more than one gene may be involved. The beauty mark does not become visible until after the first molt but can be followed through to pupation; however, it is somewhat difficult to identify in the late fourth and fifth instar larvae.

We are now in the process of determining how prevalent this mark is in wild larvae. If it is scarce, then it may be a potential marker for the F_1 program.

References Cited

McManus, M. L., and D. Twardus. 1988. Identifying Gypsy Moth Early Larval Instar. USDA, Forest Service, Northeastern Forest Experiment Station: 1 p.

Mastro, V. C., and C. P. Schwalbe. 1988. Status and potential of F_1 sterility for control of noxious lepidoptera in Modern insect control: nuclear techniques biotechnology. International Atomic Energy Agency. Vienna, Austria:15-40.

Tanner, J. A., S. E. Lane and H. M. Thatcher. 1989. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center rearing facility. APHIS Laboratory Report, October 1, 1988 - September 30, 1989: in press.

Table 1. Peak four days of adult male emergence in the Otis New Jersey strain.

	Replication					
	1		2		3	
	Peak[1/]	No. Cups	Peak	No. Cups	Peak	No. Cups
Population	41-44	966	39-42	966	38-41	966
Front Cart[2/]						
whole	41-44	483	39-42	483	39-42	483
end	41-44	105	39-42	105	38-41	105
Back Cart[3/]						
whole	41-44	483	39-42	483	38-41	483
end	41-44	105	39-42	105	38-41	105

1/ The four consecutive days with highest adult male emergence.
2/ Front cart is nearest the center room aisle. The end row is the last row of cups that face the back cart.
3/ Back cart is nearest the wall. The end row is the last row of cups that face the front cart.

Table 2. Deviation of the predicted peak four day male emergence from the actual population peak four day male emergence as affected by the sampling method.

		# days predicted peak four days emergence deviated from that of the population								
		+3	+2	+1	0	-1	-2	-3	-4	-5
Actual percentage	mean	24.2	39.7	55.8	62.4	58.2	48.7	34.7	23.0	16.0
of male emerging	±S.E.	3.8	4.0	3.8	1.6	0.6	2.1	3.8	3.6	1.7
Sampling method	# cups/sample	number of samples/day deviation n=60								
resample[1/]	8	0	2	12	16	12	7	7	3	1
continuous use[2/]	8	0	2	16	18	15	6	2	1	0
continuous use	16	1	2	12	25	18	2	0	0	0
continuous use	24	0	3	13	39	5	0	0	0	0

1/ New cups selected on each day.
2/ First day sample cups continuously used.

Table 3. Development of Otis New Jersey strain gypsy moth as affected by the geographic origin of the agar used for solidifying the B-4 diet (±1 S.E.).

Parameter	Japan (2%)	Japan (1%)	Moroccan Dark (2%)	Moroccan Light (2%)	Moroccan Washed[1/]	Moroccan Supernatant[2/]
% hatch[3/]	66.5±3.3ab	74.3±2.0a	66.2±2.5ab	61.0±3.3b	69.8±2.6ab	71.9±2.9ab
% establish	97.6±1.0a	94.7±1.7a	94.0±1.5a	94.0±1.6a	95.1±1.6a	94.3±1.5a
% pupation	71.1±20.7a	58.6±24.2bc	53.0±26.0c	53.5±24.4c	69.9±21.8ab	69.2±18.8abc
% pupal deformity						
♂	11.3±11.6a	18.0±9.4a	13.6±6.9c	20.3±18.1a	17.8±3.8a	10.6±9.3a
♀	77.7±13.5a	88.0±18.5b	92.4±5.1bc	93.5±8.3bc	96.1±6.8c	90.3±7.3bc
Pupal wt(gms)						
♂	0.71±0.01a	0.68±0.02a	0.73±0.02a	0.71±0.02a	0.72±0.01a	0.71±0.01a
♀	2.15±0.06a	2.15±0.06a	2.20±0.07a	2.13±0.07a	2.18±0.05a	2.11±0.05a
Time to 50% pupation (DT50)						
♂	29.1±0.3a	29.7±0.3a	29.6±0.4a	29.8±0.4a	28.5±0.4a	29.1±0.4a
♀	30.9±0.2a	31.2±0.2a	31.9±0.3a	30.9±0.5a	30.7±0.2a	31.2±0.3a
% adult emergence						
♂	88.7±5.0a	87.5±4.7a	89.2±7.2a	84.9±5.3a	89.4±1.0a	86.3±7.8a
♀	70.7±3.6a	65.7±10.1a	69.2±14.0a	66.8±17.9a	79.6±8.3a	72.9±19.3a
% adult deformity						
♂	22.3±3.7a	29.6±5.2a	29.4±14.9a	25.5±11.8a	7.6±2.9a	12.8±4.5a
♀	35.1±5.3a	46.8±7.0a	44.3±14.4a	42.8±20.9a	32.3±4.9a	44.7±3.3a

1/ Agar was mixed in cold water and centrifuge with the supernatant being removed.
2/ Supernatant added to diet.
3/ Means within a row not followed by same letter are significantly different ($p = .05$).

Table 4. Reproductive data and the incidence of abnormal performance syndrome in the egg masses of Otis New Jersey strain gypsy moth as affected by the geographic origin of the agar used for solidifying the B-4 diet (±1 S.E.).

Parameter	Japan (2%)	Japan (1%)	Moroccan Dark (2%)	Moroccan Light (2%)	Moroccan Washed[1/]	Moroccan Supernatant[2/]
Egg mass wt. (gms)[3/]	0.51±0.04a	0.48±0.06a	0.53±0.08a	0.53±0.06a	0.39±0.06a	0.42±0.05a
% hatch	44.6±7.2a	44.4±9.4a	35.2±10.6a	53.2±8.4a	52.3±10.1a	42.0±9.5a
% embryonation	82.9±5.3a	77.6±8.1a	71.8±9.6a	74.1±8.1a	66.4±9.6a	62.8±9.1a
% females depositing egg mass	96.7±5.7a	93.3±11.6ab	86.7±23.1ab	70.0±52.0b	96.7±5.7a	96.7±5.7a
% masses classified with APS	78.5±11.2a	70.8±11.0ab	61.1±25.5ab	56.7±37.9b	79.2±19.1a	81.5±17.0a
% masses classified as normal[4/]	21.5	29.2	38.9	43.3	20.8	18.5

1/ Agar was mixed in cold water and centrifuge with the supernatant removed.
2/ Supernatant added to diet.
3/ Means within a row not followed by the same letter are significantly different (p = .05)
4/ % normal egg masses = 100 - (% APS masses).

Table 5. Development of Otis New Jersey strain gypsy moth as affected by the substitution of wheat germ meal for wheat germ in the B-4 diet (±1 S.E.).

Parameter	wheat germ	wheat germ meal
% survival to pupation[1/]	91.7±1.9a	92.4±1.7a
Pupal wt(gms)		
♂	0.70±0.01a	0.70±0.03a
♀	2.29±0.13a	2.18±0.25a
% pupal deformity		
♂	0.0±0.0a	1.6±0.8a
♀	40.7±3.4a	71.9±13.3b
Development time to 50% pupation (days)		
♂	24.9±0.2a	24.4±0.2a
♀	27.4±0.3a	26.9±0.8a
% adult emergence		
♂	99.0±0.0a	100.0±0.0a
♀	99.0±0.0a	96.0±0.0a
% adult deformity		
♂	1.0±0.0a	2.8±1.8a
♀	5.6±0.0a	7.4±3.2a
sex ratio (♂:♀)	1.2±0.2a	1.2±0.2a

1/ Means within a row not followed by the same letter are significantly different (p = .05).

Table 6. Reproduction of Otis New Jersey strain gypsy moth as affected by the substitution of wheat germ meal in the B-4 diet (±1 S.E.).

Parameter	wheat germ	wheat germ meal
% egg masses deposited[1/]	86.7±5.8a	90.0±0.0a
Mass wt (gms)	0.65±0.05a	0.62±0.05a
% hatch	87.9±1.3a	83.5±1.7a
% embryonation	93.4±3.9a	97.3±1.1a
% masses classified as APS	0.0	0.0

1/ Means within a row not followed by the same letter are significantly different (p = .05).

Table 7. The influence of hybridization on the expression of a white, shield-shaped (beauty) mark) on the dorsal side of the metathorax segment in Otis New Jersey strain gypsy moth.

PNID[1/]	♂B x ♀B[2/]	♂N x ♀B	♂B x ♀N	♂N x ♀N
7	93.2±6.4[3/]	88.2±7.5	78.2±15.1	4.0±5.4
14	90.3±7.8	76.6±12.0	69.4±10.3	3.7±4.8
21[4/]	95.3±7.1	85.2±7.4	76.0±8.6	3.4±4.0

1/ Post neonate infest days.
2/ B = beauty mark present on metathorax segment.
N = normal markings on metathorax segment.
3/ Percentage of larvae with beauty mark (±1 S.E.).
4/ Some larvae were already pupated and were not included in the count.

Project Number: GM 86.2.5.A
Project Title: Behavioral competitiveness of F_1-sterile gypsy moth larvae under field conditions
Report Period: May 1, 1989 to September 30, 1989
Report Type: Interim
Project Leader: R. W. Hansen

Introduction

Previous reports (Hansen 1987, 1988; Mastro and Schwalbe 1986) indicated that fifth-stadium F_1-sterile gypsy moth larvae are more likely to rest under burlap bands or move off unbanded trees during the day. Untreated lab strain ("New Jersey") and feral larvae more often utilized on-tree diurnal resting sites during these experiments. For this reason, "atypical" diurnal location patterns exhibited by F_1-sterile fifth (and sixth) instars are likely a consequence of parental irradiation. I suggest that F_1-sterile fifth instars may exhibit aberrant thigmotropic or phototropic responses as a consequence of paternal irradiation, and may be less likely to "recognize" on-tree resting sites. Alternatively, older F_1-sterile larvae might exhibit greater "irritability" or increased crawling tendencies, travelling greater downward distances than their untreated counterparts before selecting diurnal resting sites.

F_1-sterile larvae used in the previous experiments were obtained from fathers irradiated with a 10 krad dose. We do not know if insects subjected to lower radiation doses (6 or 8 krad) exhibit similar location patterns. If the radiation-induced genetic damage leading to anomalous behavior(s) is dose-dependent, the use of lower paternal radiation doses may correct the "problem".

The purpose of this experiment was to compare diurnal and nocturnal location patterns exhibited by 6, 8, and 10 krad F_1-sterile fifth instars under field conditions.

Methods

In an outdoor insectary, F_1-sterile progeny from fathers treated with 6, 8 and 10 krad doses and untreated lab-strain insects were reared from egg hatch to the fifth stadium on black oak foliage (ca. 500 L_1 started for each treatment). Additional larvae of each treatment were reared in the insectary on standard artificial diet (n = 10 L_1/6 oz cup); these were transferred to foliage as needed. Feral fourth instars were collected from an infestation near Fall River, MA, and reared on oak foliage to the next stadium.

Ten black oak trees at least 8 m tall were selected from a stand in the Massachusetts Military Reservation. No resident gypsy moth egg masses or larvae were found in the area. Burlap bands 25 cm wide were placed around the bole of each tree at 1, 3, and 5 m above ground. A band was also placed at 1 m on the boles of any trees whose crowns touched those of the selected tree (generally, 2-4 additional trees). Understory vegetation and woody debris was removed within 1 m of each selected tree, and six wooden shelters (raised 15 x 20 cm plywood squares) were placed equidistantly at ca. 12 cm from each bole.

On July 6 and 7, three groups consisting of 25 fifth-stadium larvae from each treatment (6, 8, and 10 krad F_1-sterile, lab-strain, and feral) were collected and dusted with fluorescent powders identifying each treatment. Between 1100 and 1300, one group (n = 125 L_5) was released on the boles (ca. 2 m height) of each of three selected trees. In these "preliminary" releases, the trees were revisited 24 hr later, and burlap bands, wooden shelters, and other on-tree and off-tree sites examined for marked larvae.

Results and Discussion

No larvae could be located on or adjacent to one release tree after 24 hr. Four marked larvae (3.2% recovery rate) were recovered on or near each of the other two trees. Because of these low recovery rates, insufficient to detect among-treatment differences, this experiment was terminated.

Despite thorough searching of artificial and natural resting sites on and off selected trees and careful searching of oak crowns and understory vegetation, only a very few marked larvae could be recovered only 24 hr after larval releases. Such "losses" were unprecedented in previous experiments and hence unexpected. The fate of unrecovered larvae was unknown. Traces of the marking powders were found on oak boles, branches, and foliage, indicating that marked larvae had indeed dispersed from release locations. Unrecovered larvae could have been consumed by birds or small mammals, dispersed to distant trees and/or off-tree sites, or simply moved to crown peripheries on selected trees, where I could not see them.

This experiment may be repeated during the summer of 1990, using the caged-tree system employed in earlier experiments (Hansen 1988).

REFERENCES

Hansen, R.W. 1987. Comparative location patterns of late-stadium gypsy moths. pp. 111-117 in 1987 USDA-APHIS Otis Methods Development Center Progress Report.

Hansen, R.W. 1988. Behavioral competitiveness of F_1-sterile gypsy moth larvae under field conditions. pp. 173-190 in 1988 USDA-APHIS Otis Methods Development Center Progress Report.

Mastro, V.C. and C.P. Schwalbe. 1986. Comparative larval behavior of gypsy moths. pp. 106-110 in 1986 USDA-APHIS Otis Methods Development Center Progress Report.

Project Number: GM 86.2.5.B
Project Title: Mating competitiveness of F_1-sterile gypsy moth adults
Report Period: October 1, 1988 to September 30, 1989
Report Type: Interim
Project Leaders: V.C. Mastro, A. Pellegrini-Toole, R.W. Hansen

Introduction

Previous investigations (Hansen 1988, Mastro *et al* 1988) have shown that F_1-sterile, untreated lab-strain, and feral gypsy moth males and females may mate more than once in field cage environments. For both sexes, F_1-sterile mating patterns do not differ from those of untreated adults. In general, females initially mated by F_1-sterile males were as likely to remate as those mated by normal lab-strain or wild males. Remating by females generally increased with increased male:female ratios. Finally, preliminary data indicate that males mating last (i.e. closest to oviposition) will achieve fertilization precedence.

Field cage experiments conducted in 1989 were designed to provide additional supporting evidence for these general observations. Male and female mating patterns under varying male:female and overflooding (sterile:fertile) ratios would be examined, and replicated in two types of field cage to describe "test arena" effects. Specifically, we wished to further quantify fertilization precedence in multiply-mated females and its implications for F_1-sterile release programs.

Methods

In an outdoor insectary, F_1-sterile (10 krad paternal radiation dose), untreated lab-strain ("NJ"), and feral (collected as egg masses from northern Virginia) gypsy moth larvae were reared from hatch to pupation on black oak foliage. Larvae were provided with fresh foliage every two or three days as required. Supplemental collections of feral late-stage larvae and pupae were made in eastern Maine and southeastern Massachusetts.

Pupae were collected from rearing cages daily, sexed, and placed, in groups of 10-40, in 16-oz paper cups. Collected pupae were held under ambient environmental conditions until adult eclosion. Eclosed adults of desired treatments were collected daily and dusted with fluorescent powders or numbered on the right forewing prior to release. Adults employed in these experiments were generally less than 24 hrs. old; occasionally, we used males that were 24-48 hrs. old.

Four cages, two "small" (3.5x3.5x1.8 m) and two "large" (7x7x5.5 m) fine-mesh screen cages were erected in a pine-oak forest. Most vegetation was removed from inside the cages. Temperature and humidity were continuously monitored at a nearby electronic hygrothermograph, and hourly temperature readings were made within the cages while studies were conducted.

Fifteen adult releases were conducted from July 25 to August 8, 1989, each initiated between 0830 and 1000 and completed by 1600 (EDT). Feral females (VA, ME, or MA) were released on cage walls (small cages) or on a 1.5 m wooden post (located centrally in the large cages). Marked males dispersed from holding cups placed on the floor of the cages (small cages) or were scattered about the periphery of the cage (large cages). Males were released to achieve overflooding ratios of 1:1 (n=20:20), 3:1 (n=30:10), or 4:1 (n=20:5) (F_1-sterile: feral males), M:F ratios of 1:1 (n=40:40), 3.3:1 (n=40:12), or 10:1 (n=40:4), and various combinations thereof. In some releases, untreated lab-strain (NJ) males were substituted for F_1-sterile males. The actual overflooding and M:F ratios attained often differed slightly from "targets" depending on the daily availability of males from a given treatment.

Released females were observed continuously and any matings recorded. Matings were considered to begin when genital coupling was initially observed. The treatment (and number) of the male involved and the initiation and completion (time EDT) were recorded for each female's mating(s). From these data, duration of, and refractory times between, mating bouts were calculated. In the small cages, the time at which a female initiated oviposition was recorded, and egg masses were collected. Collected egg masses were held in the laboratory, and embryonation and hatch rates determined after ca. 170 days in cold storage (5°C).

Results

Egg mass analyses have shown high embryonation (>80%) and hatch rates (>50%) for matings involving F_1-sterile males. Expected embryonation and hatch rates are <20% and <1%, respectively, for matings involving an F_1-sterile (10 krad) father. Thus, either our released F_1-sterile males received an inadequate paternal irradiation dose and were, in fact, not sterile, or treatments were inadvertently mislabelled and/or combined during larval rearing. We feel that this latter scenario is much more likely. For this reason, we can have no "confidence" in the identity of any males employed in this study, and data derived from these experiments are probably erroneous. Analysis of these experiments has been terminated.

Laboratory and field cage experiments will be continued in FY 1990. We will concentrate on "quality control" during the rearing of experimental material.

LITERATURE CITED

Hansen, R.W. 1988. Behavioral competitiveness of F_1-sterile gypsy moth adults under field conditions. pp. 191-200 in 1988 Otis Methods Development Center Progress Report. 288 p.

Mastro, V.C., A. Pellegrini-Toole, R.W. Hansen, and C.P. Schwalbe. 1988. Competitiveness of gypsy moth adults. pp. 209-220 in 1988 Otis Methods Development Center Progress Report. 288 p.

Project Number: GM 87.2.2
Project Title: Sterile Male Demonstration Project, Gates County, North Carolina
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leaders: V. C. Mastro, A. Pellegrini-Toole, C. P. Schwalbe, W. Wescott
Cooperators: H. Singletary, K. Wilson

F_1 egg mass releases (approximately 100,000/year) were made in the Gates County site in 1987 and 1988. The release site can be characterized as approximately 20 acres in size bordering both sides of a road. The release site is on high ground, artificially created when the road bed was constructed, and surrounded by swamps. Within the release site, the majority of trees are small (2-6 in DBH) red maples with scattered willows (apparently the host which most of the wild population of gypsy moths was utilizing). As described in earlier reports, the gypsy moth population at this site was never adequately defined either in terms of density or distribution.

Table 1 presents the final results of 1988 and restates the 1987 results. Although immature sterile:fertile ratios (i.e., chromosome analysis ~ 23:1 and mating evaluation ~ 25:1) indicated good overflooding ratios, the post-season egg mass evaluation indicates that the adult mating ratios were very skewed in favor of fertile wild insects. Even the sampling of immature stages did not provide overflooding estimates as high as estimated (i.e., 58:1 - 2666:1) and the sterile:wild ratio generally deteriorated throughout the season.

An examination of the relative development of F_1 and wild collected immatures provides an explanation for the high proportion of post-season egg masses that had a wild male parent 96.7% (Table 1). Figures 1a-1f present the life stages of males at collection which were later determined to be wild or sterile F_1s. Generally, for all life stages, development of F_1 male insects lagged 1-2 weeks behind development of wild males. Although a small proportion of F_1 males reached the pupal stage in synchrony with wild males, the majority did not pupate until 1-2 weeks after wild insects (Figure 1f). This asynchrony of development coupled with the deterioration in numbers of the F_1 population provides reasonable explanations for the poor suppression. Apparently F_1 female development was nearly synchronous with wild female development (Figures 1g-1m). F_1 and wild females were very synchronous in reaching the pupal stage. Post-season egg mass evaluation indicates that overflooding rates, although poor, were relatively better for females than for males.

In summary, the asynchrony in development of wild and F_1 males contributed to the low sterile:fertile mating ratios in post-season egg masses. Contributing to this poor overflooding rate was a general decline in the proportion of F_1 males throughout the larval development period. The general decline in number of F_1 males may be related to asynchrony in development. F_1 egg hatch was apparently timed properly (Figure 2). Possible explanations for the asynchrony in larval development are: (1) released F_1s (or laboratory strain) were incapable of responding to the relatively high temperatures at this site during the development period; (2) released insects were displaying characteristics of the delayed development phenomenon known as straggling.

Table 1. Results of monitoring sterile F_1:wild overflooding ratios in Gates County, North Carolina.

	1987			1988		
Number of F_1 egg masses released	100,300			100,000		
Expected S:F male ratio	None			58:1 - 2666:1[3/]		
Monitoring technique	No. samples	Ratio S:F	% fertile	No. samples	Ratio S:F	% fertile
Male chromosome analysis[1/]	147	147:0	0.0	71	22.7:1	4.2
Male mating evaluation[1/]	623	28.7:1	3.4	777	24.9:1	3.9
F_1 monitor female egg mass evaluation	58[2/]	3.3:1	23.3	32	31:1[2/]	3.1
Female mating evaluation[1/]	484	14.6:1	6.4	570	4.8:1	17.4
Post-season egg mass evaluation	1196	12:1	7.7	989	0.7:1	58.3
Male parent	880	x:1		880	0.03:1	96.7
Female parent	966	6.8:1		1004	0.6:1	61.9
Number of males trapped	2534			2966		

1/ Insects collected as immatures (larvae or pupae) and evaluated at the Otis Methods Development Center.
2/ An additional 178 (1987) and 339 (1988) monitor females placed could not be used for establishing a mating ratio: 233 (1987) and 311 (1988) failed to oviposit; 48 (1987) and 27 (1988) produced egg masses with all unembryonated eggs; and 7 (1987) and 1 (1988) produced egg masses that had degrees of embryonation that statistically could not be separated from the two possible mating types.
3/ Based on high and low ratios observed and male trap catches = 20% of male population in 1987 and projected number of wild egg masses in the spring of 1988.

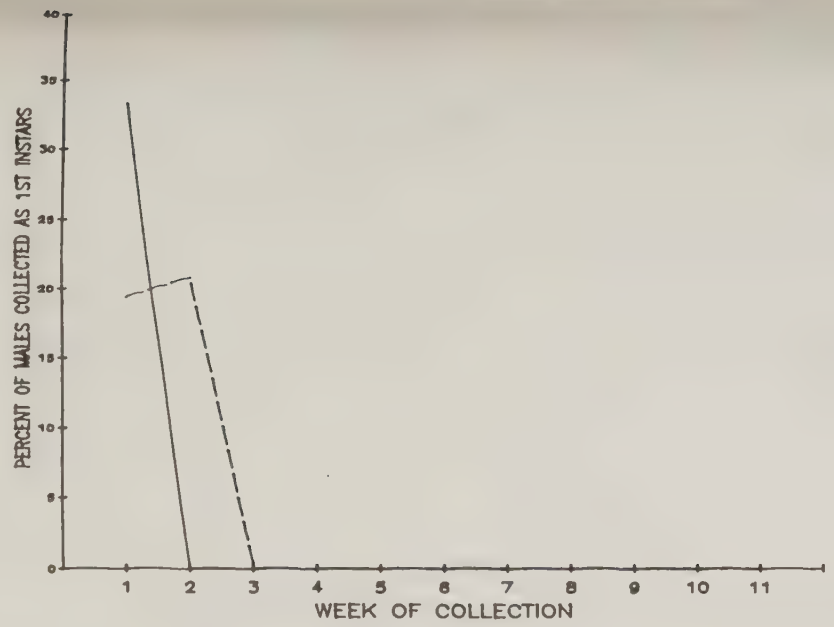


Figure 1a

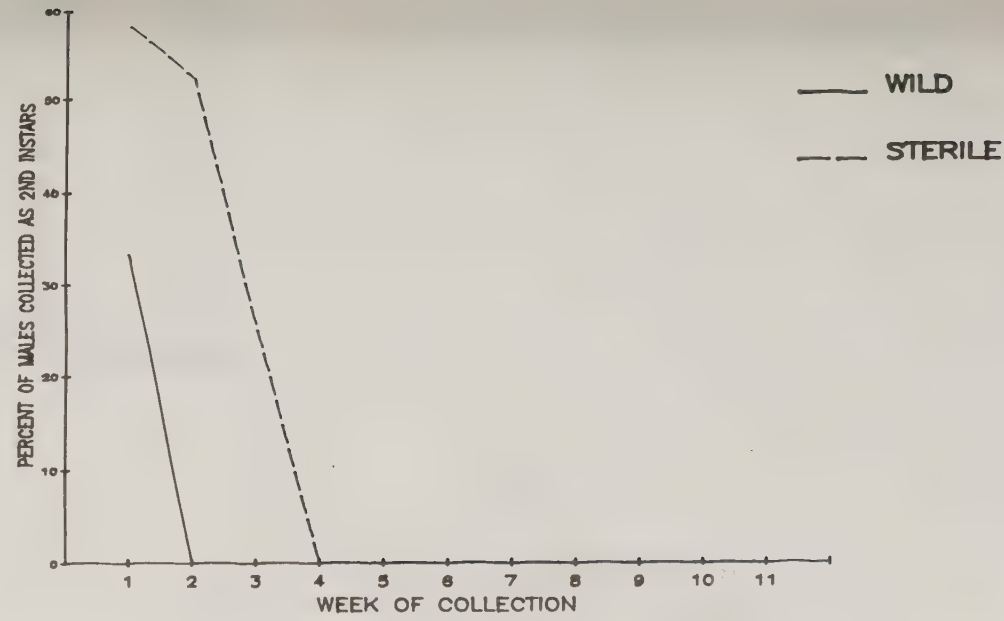


Figure 1b

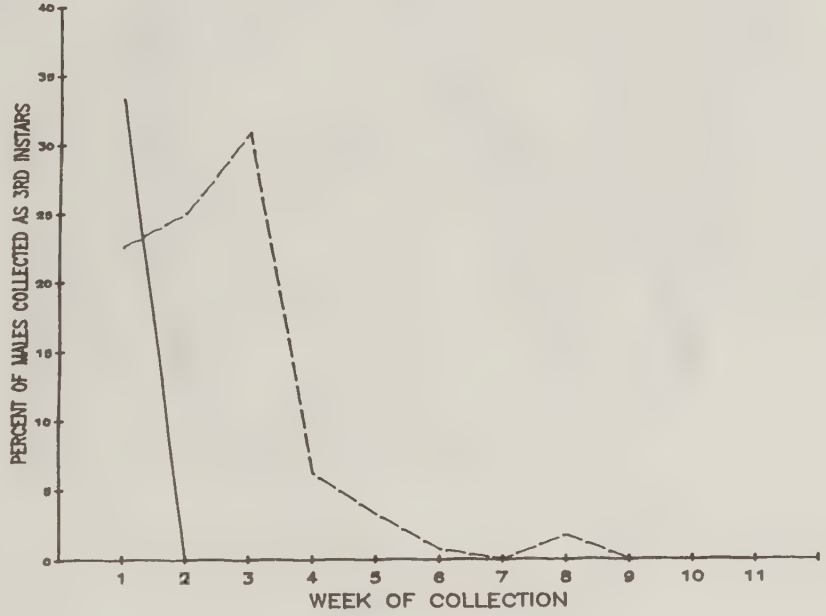


Figure 1c

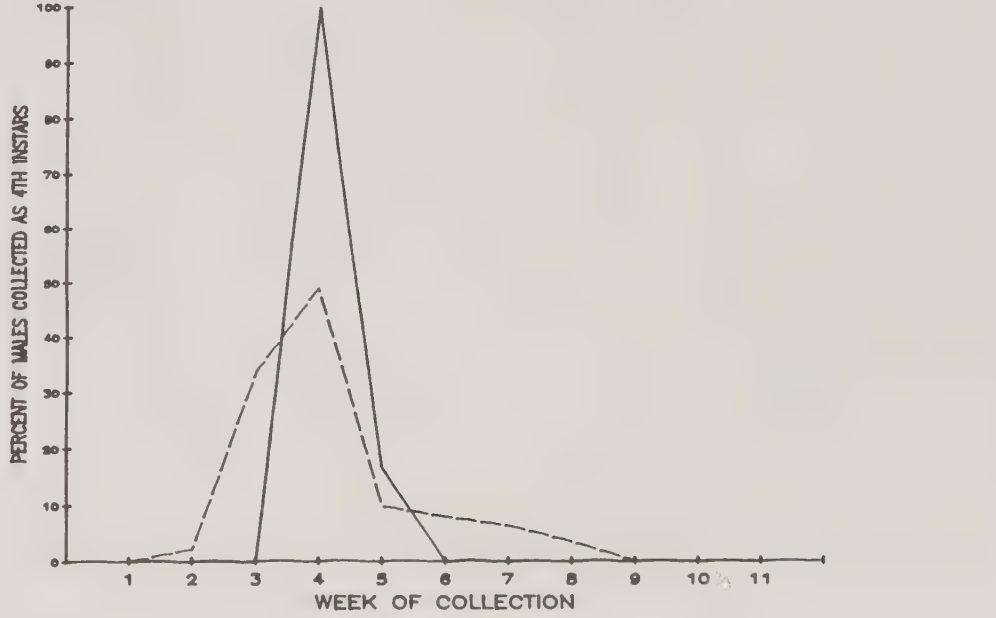


Figure 1d

Synchrony of male development
Gates Co., NC 1988

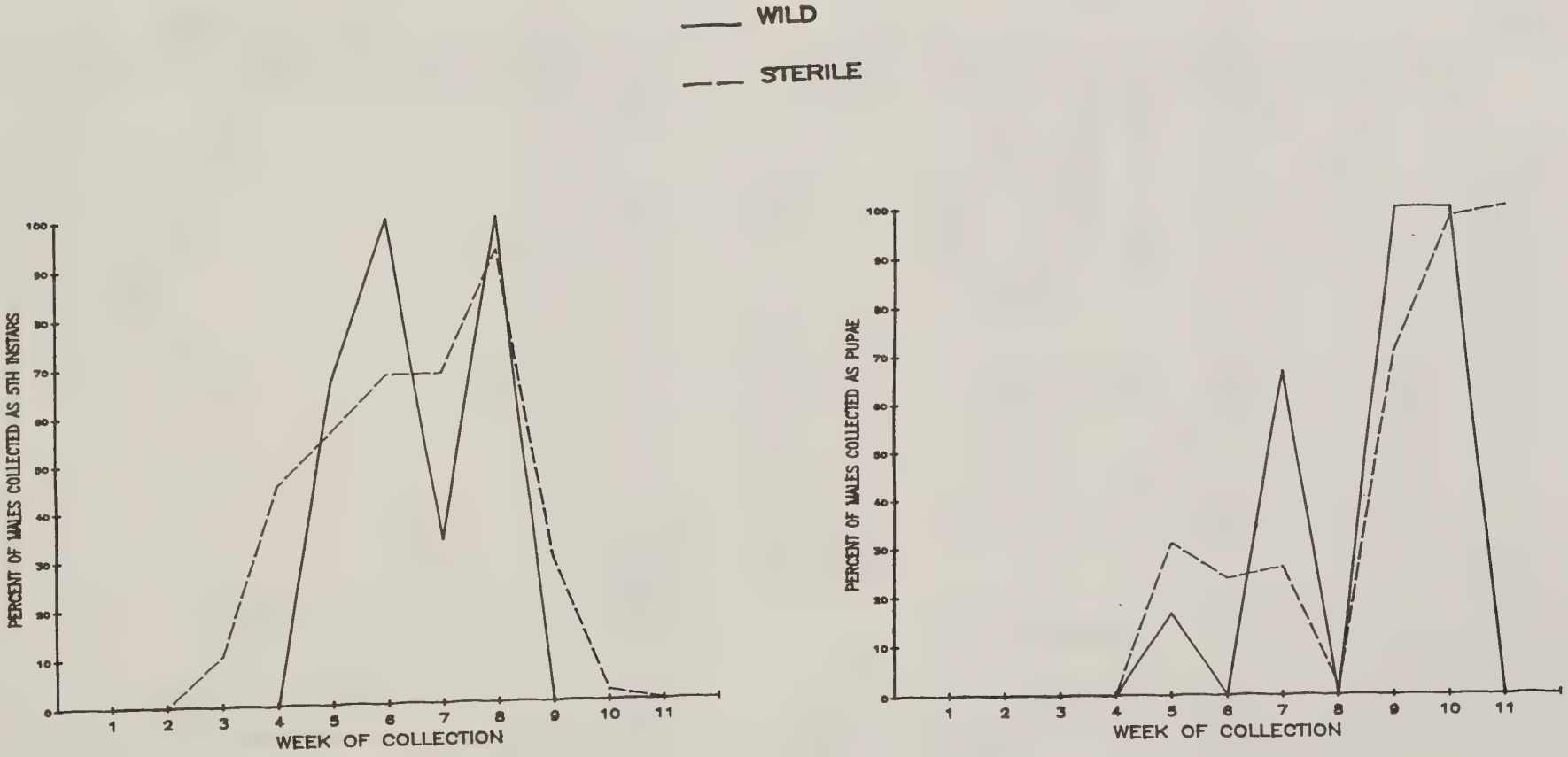


Figure 1e

Figure 1f

Synchrony of male development
Gates Co., NC 1988

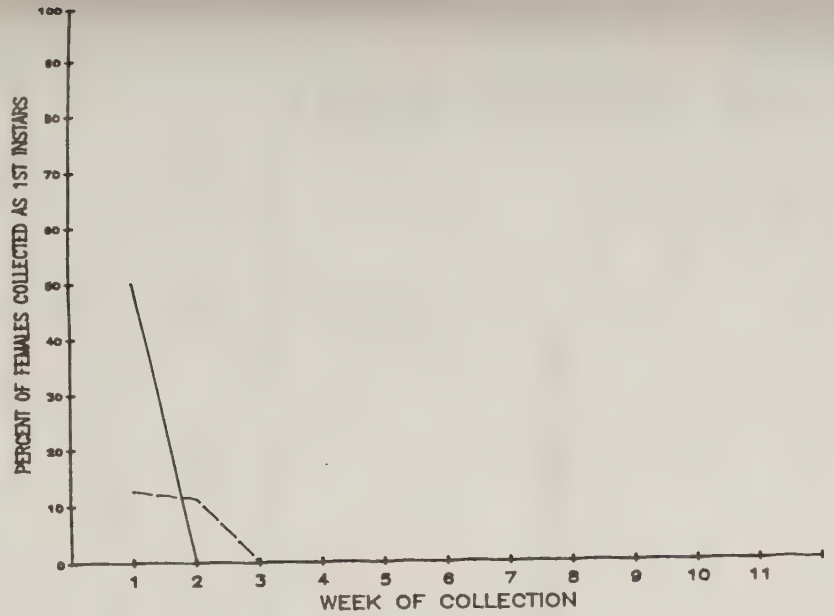


Figure 1g

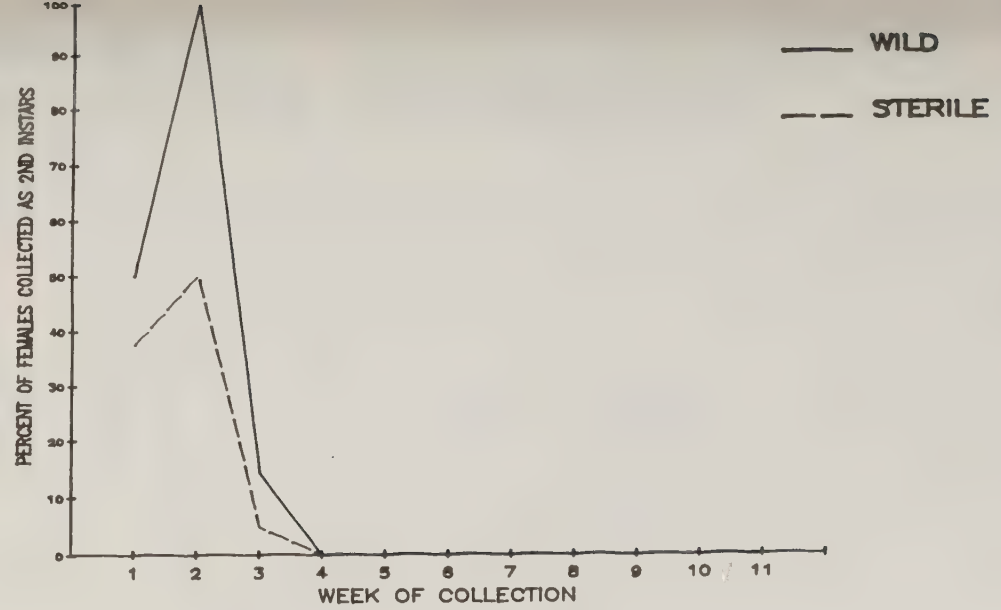


Figure 1h

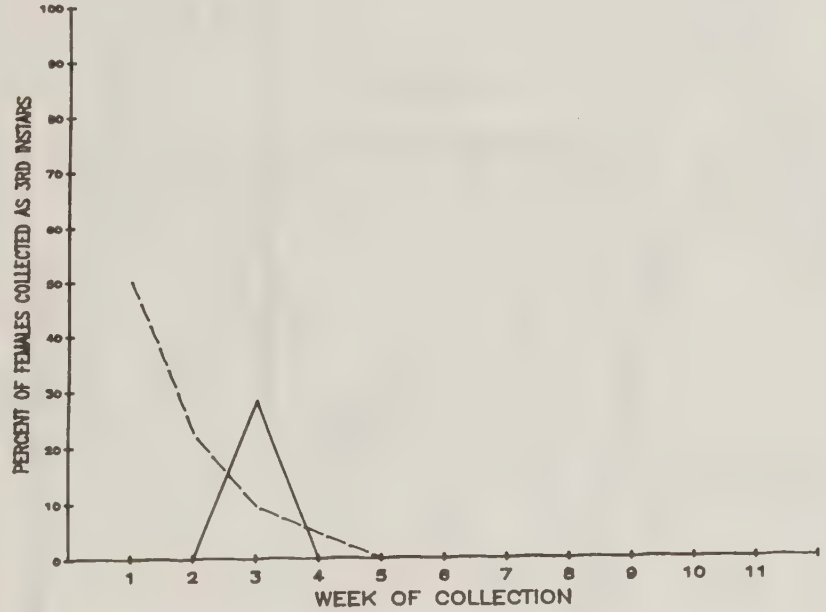


Figure 1i

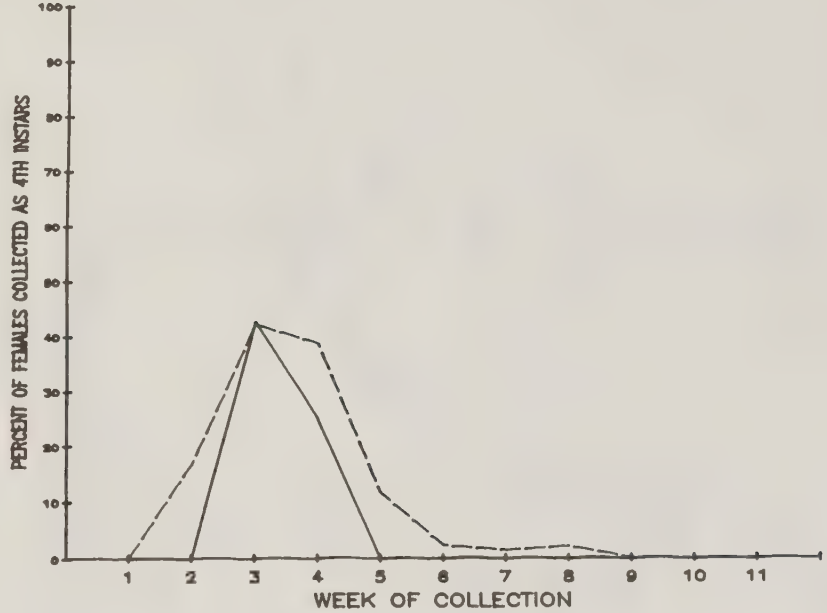


Figure 1j

Synchrony of female development
Gates Co., NC 1988

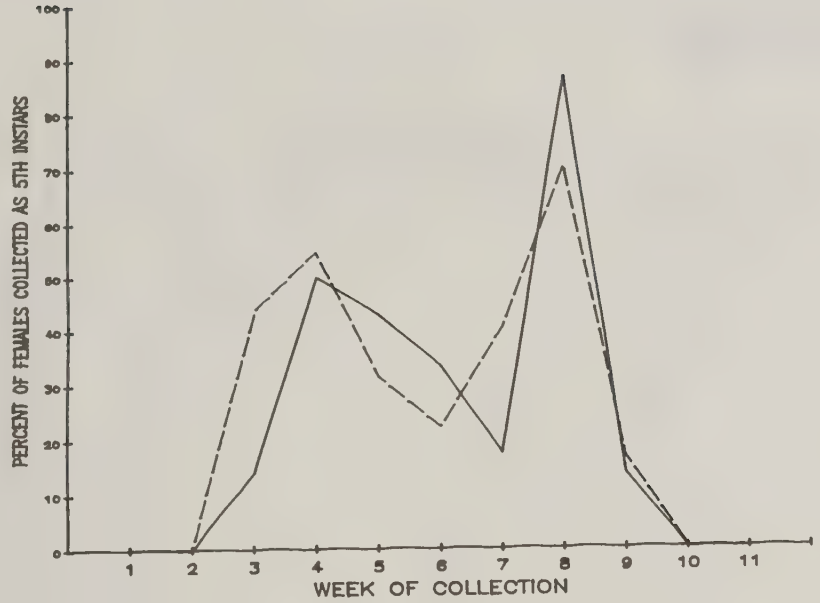


Figure 1k

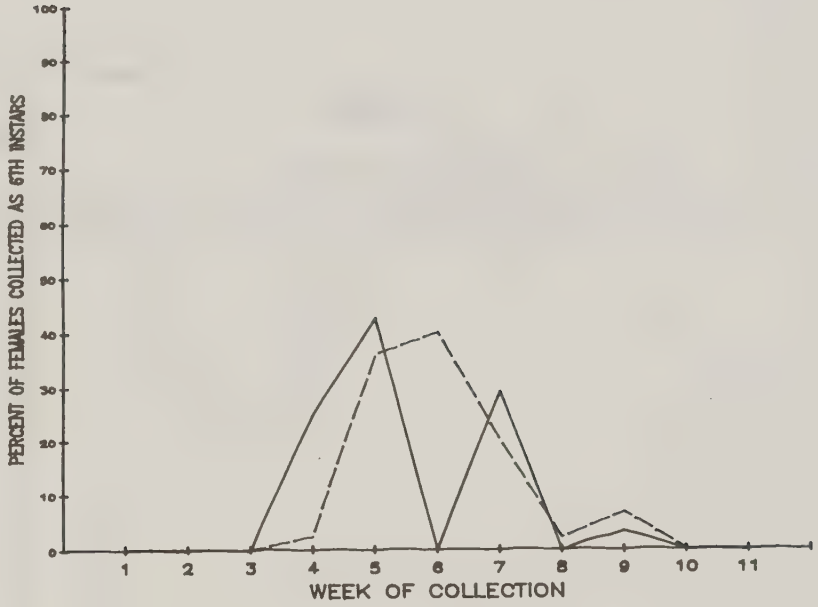


Figure 1l

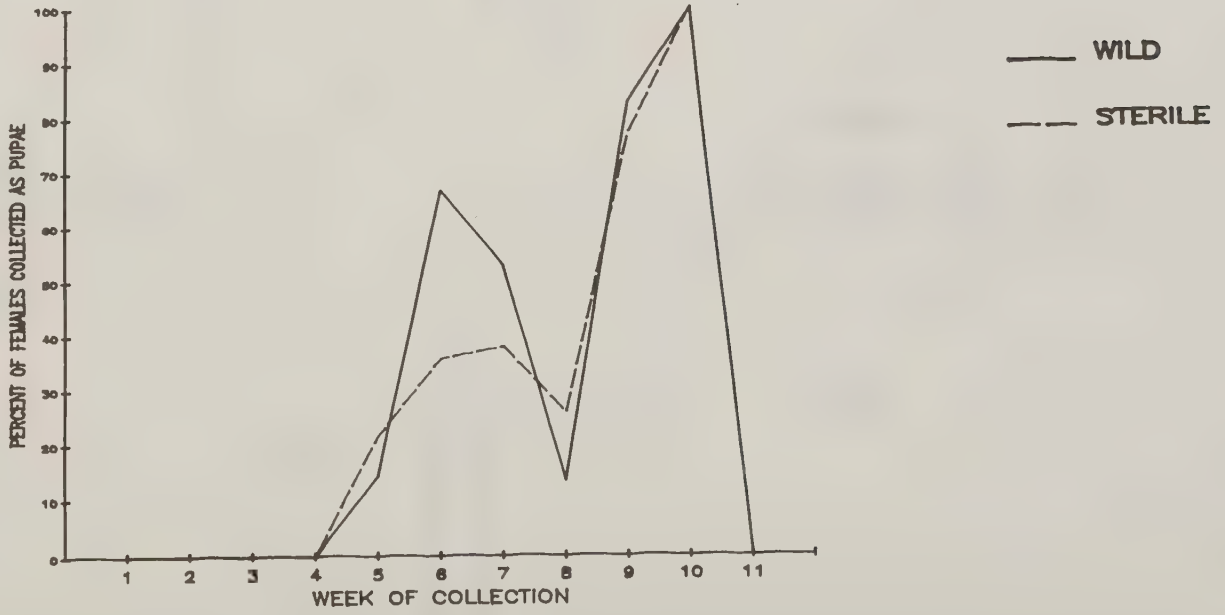
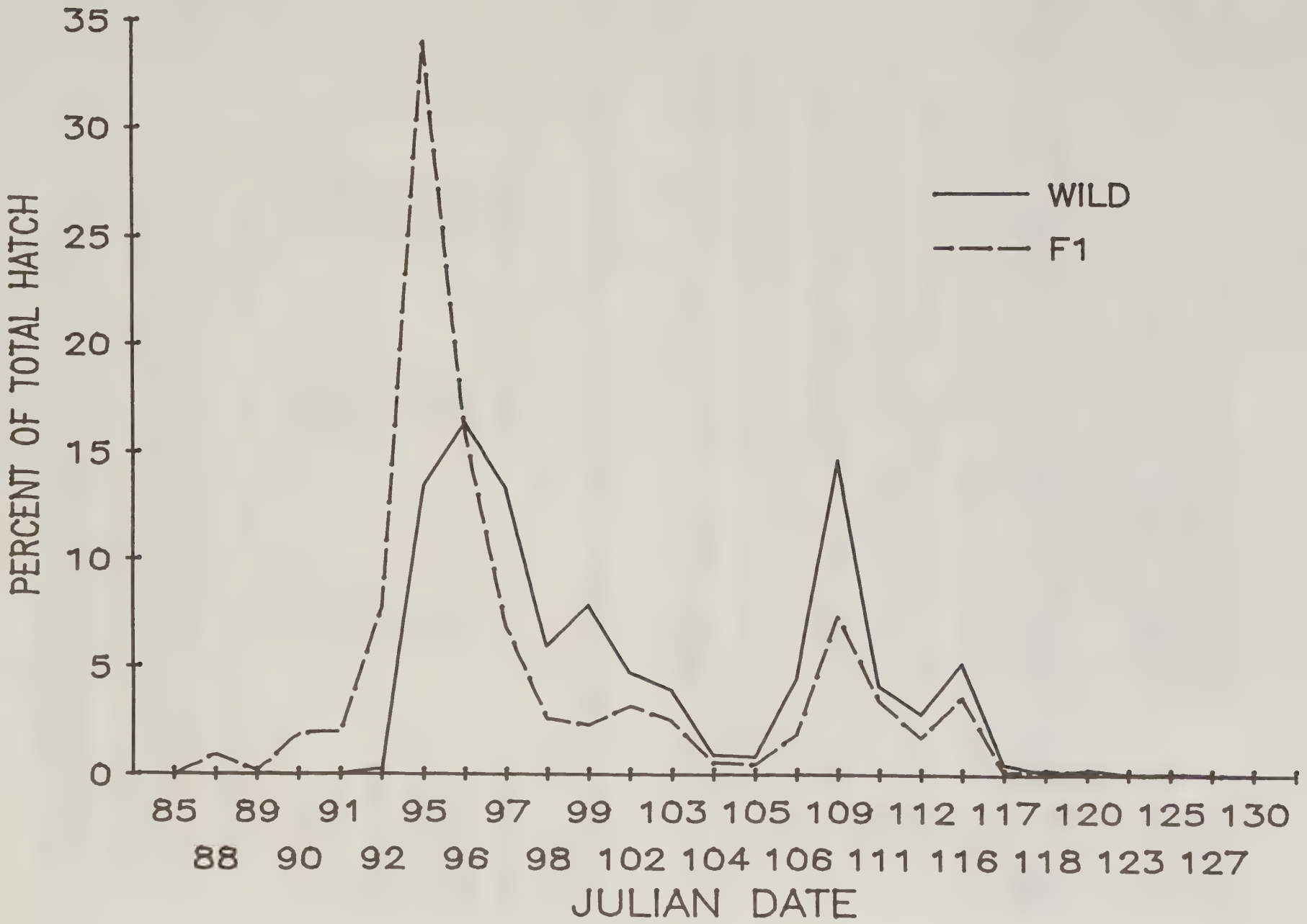


Figure 1m

Synchrony of female development
Gates Co., NC 1988

Figure 2.


1988 GATES CO. EGG HATCH SYNCHRONY
PERCENT OF TOTAL HATCH
35
30
25
20
15
10
5
0
WILD
F1
85 89 91 95 97 99 103 105 109 112 117 120 125 130
88 90 92 96 98 102 104 106 111 116 118 123 127
JULIAN DATE

Project Number: GM 88.2.1
Project Title: A New Large-Capacity Gypsy Moth Trap Design
Report Period: October 1, 1988 - September 30, 1989
Report Type: Preliminary
Project Leaders: E. C. Paszek, V. C. Mastro

In 1988 a new large-capacity trap design, the Cono-cup, was bioassayed and it compared favorably to the standard milk carton trap. This trap is readily assembled in the field and stores in a compact space; 300 nesting cups fit into a 23" x 16.5" box.

The following 7 large-capacity traps of various designs were compared:

1. Cono-cup L.H. -- large hood overhang extending 2.75" above 8 (1 x 5/16") entry ports
2. Cono-cup N.H. -- no hood overhang above 8 entrance ports
3. Cono-cup L.H. & B. -- large hood overhang extending 2.75" above 8 entrance ports; a gallon-size plastic baggie
4. Cono-cup S.H. -- small hood overhang extending 1.5" above entrance ports
5. USDA Milk Carton -- standard 1/2 gallon size gypsy moth trap with a 2/75" overhang extending above 8 (1 x 5/16") entrance ports
6. Hercon experimental milk carton -- similar in design to USDA standard milk carton
7. Briese Diamond -- diamond shaped trap with gallon size plastic bag to bottom, no overhang above entrance ports
8. Cono-cup R.E.P. -- a single 1" diameter round entrance port cut in the apex pour spout. Spout in closed position forms a 1.5" gable above the recessed entrance port

Mean numbers of males per observations captured in traps of various designs in Jefferson County, Pennsylvania, July 24-29, 1989[1/]

Trap design[2/]	n	mean[3/]	Std. error
Cono-cup L.H.	20	72.1 a	14.95
Cono-cup N.H.	20	70.0 a	18.67
Cono-cup L.H. & B.	20	69.3 a	15.23
Cono-cup S.H.	20	60.9 ab	14.50
USDA milk carton	20	51.1 ab	10.67
Hercon	20	50.4 ab	13.66
Briese Diamond	20	39.2 b	9.99
Cono cup R.E.P.	20	10.3 c	1.96

1/ Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test

2/ Cono-cup L.H. -- large hood (2.75" overhang); Cono-cup N.H. -- no hood; Cono-cup L.H. & B. -- large hood and bag (hood 2.75" overhang) and catch bag fastened to bottom; Cono-cup S.H. -- small hood (1.5" overhang); standard USDA milk carton trap; Hercon -- experimental milk carton trap; Briese Diamond (diamond shaped trap with plastic catch bag attached to bottom); Cono-cup R.E.P. one 1" diameter round entry under folded section of top.

3/ Traps were arranged for testing in a randomized complete block design with 50m spacing between lines (blocks) and between traps within a line. Traps were read and randomized daily. Analysis of variance was performed on transformed data ($\sqrt{\text{males}} + 0.5$); actual means are present.

Results

There was no significant difference between the 3 Cono-cup variations L.H., L.H.& B., and N.H. They captured more moths than the standard milk carton trap (not signficant; P=.05). The S.H. Cono-cup and the Hercon trap also captured males in numbers not significantly different from that the standard USDA milk carton. The Briese Diamond-shaped trap captured fewer (but not signficantly) males than the standard. The Cono-cup R.E.P. with one entrance port at the apex was designed to catch smaller numbers of moths in moderate to heavy infestations (i.e., a monitoring trap). In this study, the Cono-cup R.E.P. capture rate was approximately 15% of the standard Hercon trap. This lower capture rate is in the range of a desired trapping level for monitoring gypsy moth populations of various densities. These varied Cono-cup trap designs should be retested in the new larger version of the Cono-cup, which is made of heavier plasticized paper.

Project Number: GM 88.2.5
Project Title: Sterile Male Demonstration Project, Fort Collins, Colorado
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leaders: V. C. Mastro, A. Pellegrini-Toole, C. P. Schwalbe
Cooperators: O. Barham, D. Leatherman

Preliminary results of this project were reported in the last annual report (GM 88.2.5, pp. 240-242). Table 1 summarizes the final results of this study. As was stated in the last report, all evaluation techniques indicated high sterile:fertile overflooding ratios. Post-season egg mass evaluation not reported earlier indicates that all egg masses (n=3) were sterile. The sterile: fertile male mating ratio, however, was very low (1:2). A number of possible reasons for this skewed ratio were offered in the report on an F_1 release in North Carolina (GM 87.2.2).

Five males were caught in the sterile male release area (25 traps/square mile) in 1989. All males were single captures. Two additional males were captured outside the area. The reduction in males trapped prior to release in 1987 (n=77) to post-release 1989 (n=5) indicates the 1988 release had a suppression effect on the native population. It should be noted that this decrease in males captured can partially be attributed to different trap densities used in the two years (1986 = 2 traps/acre; 1989 = 1 trap/25 acres).

Table 1. Results of monitoring sterile F_1:wild overflooding ratios in Fort Collins, CO, 1988.

Technique		n	F_1:wild	% fertile
Male chromosome analysis		226	224:2 (112:1)	0.9
Male mating evaluation		923	56.7:1	1.7
F_1 monitor female egg mass evaluation		112	111:1	0.9
Female mating evaluation		504	15.8:1	6.0
Post season egg mass evaluation		3	3:0	0.0
F_1:wild male ratio			1:2	66.7
F_1:wild female ratio			2:0	0.0
Males trapped	core area	117		
	associated	26		
	Total	143		

This target population, unlike some others treated using the F_1 techniques, had fewer host trees for establishment of F_1s. In addition, the native population had, in retrospect, an extremely clumped distribution. If the native insect distribution was known before release, the distribution of F_1 might have been planned more effectively. Finally, the reasons for the skewed male mating ratios established using post-season egg mass data need to be resolved. The reason for the skewed ratio in the Gates County, North Carolina site appeared to be an asynchrony in native sterile male development. Because of the few native males in the Fort Collins population, the developmental data is too scanty to support this conclusion.

Project Number: GM 88.3.1
Project Title: Gypsy Moth Mass Rearing FY89
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leader: G. Bernon

During FY89, gypsy moth colony production was done on a weekly basis following the laboratory life cycle as outlined in Figure 1. A laboratory generation is therefore 251 days, or about 35 weeks. As a result, egg masses produced after January 1989 were not available for use until FY90. A timetable for planning production levels is shown in Figure 2. Unfortunately, it became apparent early in the fiscal year that the long life cycle made it impossible to plan for the production levels needed in nine months when any subcolony would be ready to use. Only egg masses produced during the first quarter of the fiscal year (Oct.-Jan.) would be available during that same fiscal year. A schedule for rearing production was established based on projected needs. The same schedule is planned for FY90 (Figure 3).

A tray consists of 30 rearing containers and is the standard unit referred to in Figure 3. The actual production was measured by counting egg masses produced prior to placing the masses into the cold temperature (Step 5 of Figure 1). This count provided an opportunity to establish an inventory which was started at the beginning of the fiscal year (Figure 4).

Also, an effort was made to standardize production by following the exact schedule and establishing colony production as the priority project. The tendency was to emphasize the work that would have immediate results (i.e., daily production) and not colony maintenance, which would not be available for approximately nine months. This practice was started at the end of the first quarter of Fiscal Year 1989 (approx. subcolony 12, Figure 5).

When colony cohorts were removed from the cool chamber the following August, the quality of weekly subcolonies dramatically improved. The quality can be measured by the mean larval stage of an egg mass subsample when the larvae are 14 days old (Figure 5). This measurement (MLS) is a routine quality control evaluator (see Tanner, 1985 Annual Report). Subcolonies produced earlier, during the first quarter of FY90, suffered from poor growth and development, now referred to as Abnormal Performance Syndrome (APS). The presence of APS during the first quarter of FY90 is clearly shown by the low mean larval stage readings in Figure 5. Over 35 subcolonies produced in FY90 have now completed the laboratory life cycle and have been free of APS. Consequently, all subcolonies now in inventory were produced from APS-free parental stock. Unfortunately, there has been no correlation in the past between lineage and APS.

Figure 5 does, however, provide a comparison between a period when APS adversely impacted on production, and APS-free production during the last three quarters of FY90. Note that in Figure 5 the transition from APS-influenced production (approximately subcolonies 1-12) to the absence of APS (approximately subcolonies 24-46) was gradual and resulted in intermediate-quality subcolonies (approximately 13-23) for each of the subcolonies in Figure 5. The two measurements that were taken (MLS, number of egg masses) were separated by six months. Although it is possible to quantify production (number of egg masses produced) after only two months, the MLS reading is not taken until almost six months later when eggs are removed from chill. Unfortunately, determining the presence of APS in a subcolony is now dependent on the MLS reading, but it may be possible to use data collected earlier in the laboratory life cycle to predict APS.

FIGURE 1:

GYPSY MOTH - MASS PRODUCTION TIMETABLE

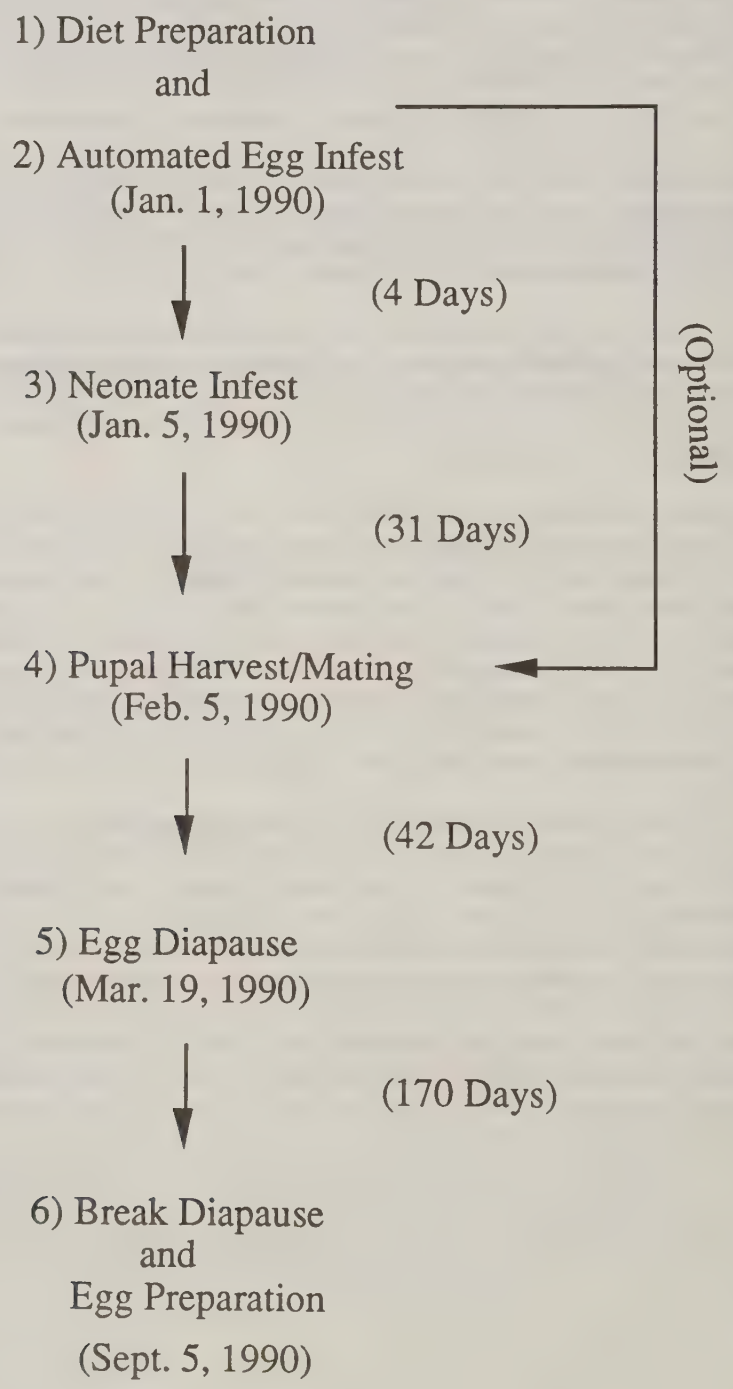


Figure 2.

WEEK	PARENT INFEST DATE JULIAN DAY	PARENT INFEST DATE CALENDAR DAY	DIAPAUSE DAY 174 JULIAN DAY	DIAPAUSE DAY 174 CALENDAR DAY	WEEK	
1	1	Jan 1	251	Sept 8	36	
2	8	Jan 8	258	Sept 15	37	
3	15	Jan 15	265	Sept 22	38	
4	22	Jan 22	272	Sept 29	39	
5	29	Jan 29	279	Oct 6	40	
6	36	Feb 5	286	Oct 13	41	
7	43	Feb 12	293	Oct 20	42	
8	50	Feb 19	300	Oct 27	43	
9	57	Feb 26	307	Nov 3	44	
10	64	Mar 5	314	Nov 10	45	
11	71	Mar 12	321	Nov 17	46	
12	78	Mar 19	328	Nov 24	47	
13	85	Mar 26	335	Dec 1	48	
14	92	Apr 2	342	Dec 8	49	
15	99	Apr 9	349	Dec 15	50	
16	106	Apr 16	356	Dec 22	51	
17	113	Apr 23	363	Dec 29	52	
18	120	Apr 30	5	Jan 5	1	Succeeding year
19	127	May 7	12	Jan 12	2	
20	134	May 14	19	Jan 19	3	
21	141	May 21	26	Jan 26	4	
22	148	May 28	33	Feb 2	5	
23	155	Jun 4	40	Feb 9	6	
24	162	Jun 11	47	Feb 16	7	
25	169	Jun 18	54	Feb 23	8	
26	176	Jun 25	61	Mar 2	9	
27	183	Jul 2	68	Mar 9	10	
28	190	Jul 9	75	Mar 16	11	
29	197	Jul 16	82	Mar 23	12	
30	204	Jul 23	89	Mar 30	13	
31	211	Jul 30	96	Apr 6	14	
32	218	Aug 6	103	Apr 13	15	
33	225	Aug 13	110	Apr 20	16	
34	232	Aug 20	117	Apr 27	17	
35	239	Aug 27	124	May 4	18	
36	246	Sept 3	131	May 11	19	
37	253	Sept 10	138	May 18	20	
38	260	Sept 17	145	May 25	21	
39	267	Sept 24	152	Jun 1	22	
40	274	Oct 1	159	Jun 8	23	
41	281	Oct 8	166	Jun 15	24	
42	288	Oct 15	173	Jun 22	25	
43	295	Oct 22	180	Jun 29	26	
44	302	Oct 29	187	Jul 6	27	
45	309	Nov 5	194	Jul 13	28	
46	316	Nov 12	201	Jul 20	29	
47	323	Nov 19	208	Jul 27	30	
48	330	Nov 26	215	Aug 3	31	
49	337	Dec 3	222	Aug 10	32	
50	344	Dec 10	229	Aug 17	33	
51	351	Dec 17	236	Aug 24	34	
52	358	Dec 24	243	Aug 31	35	

Figure 3. Fiscal Year 1990 Schedule for Rearing Production

Week Number	Beginning Date		Number of Trays to be Neonate infested
	Julian	Calendar	
1	274	Oct 1	24
2	282	Oct 9	24
3	289	Oct 16	24
4	296	Oct 23	21
5	303	Oct 30	21
6	310	Nov 6	21
7	317	Nov 13	40
8	324	Nov 20	40
9	331	Nov 27	40
10	338	Dec 4	40
11	345	Dec 11	40
12	352	Dec 18	40
13	359	Dec 25	40
14	1	Jan 1	40
15	8	Jan 8	40
16	15	Jan 15	40
17	22	Jan 22	37
18	29	Jan 29	22
19	36	Feb 6	20
20	43	Feb 13	20
21	50	Feb 20	20
22	57	Feb 27	28
23	64	Mar 6	26
24	71	Mar 13	28
25	78	Mar 20	25
26	85	Mar 27	23
27	92	Apr 3	23
28	99	Apr 10	23
29	106	Apr 17	23
30	113	Apr 24	23
31	120	May 1	23
32	127	May 8	23
33	134	May 15	23
34	141	May 22	23
35	148	May 29	23
36	155	Jun 5	23
37	162	Jun 12	23
38	169	Jun 19	23
39	176	Jun 26	23
40	183	Jul 3	23
41	190	Jul 10	23
42	197	Jul 17	23
43	204	Jul 24	23
44	211	Jul 31	23
45	218	Aug 7	23
46	225	Aug 14	24
47	232	Aug 21	24
48	239	Aug 28	24
49	246	Sept 4	24
50	253	Sept 11	25
51	260	Sept 18	25
52	267	Sept 25	24

Figure 4. Egg inventory for fiscal year 1989.

Status at end of Fiscal Year (October 1, 1989)					
Subcolony	Harvest and mate date	Diapause date	Number egg masses	% MLS>2.01	Use Date
1	288	328	580	8	136
2	293	335	942	26	143
3	302	344	42	0	152
4	309	351	449	4	159
5	316	356	0	0	164
6	323	3	896	0	177
7	330	11	423	0	185
8	337	11	1487	22	185
9	344	16	1248	19	190
10	351	24	1138	11	198
11	358	34	639	5	208
12	365	40	53	1	214
13	4	46	939	0	220
14	10	52	834	64	226
15	18	60	1132	41	234
16	23	65	605	73	239
17	32	74	1032	55	248
18	38	80	2263	37	254
19	44	86	2072	21	260
20	53	95	2518	65	269
21	59	101	3033	43	275
22	62	104	3618	94	278
23	67	109	3563	93	283
24	74	116	1983	90	290
25	82	124	3190	100	298
26	87	129	2369	50	303
27	94	136	2063	95	310
28	101	143	3924	95	317
29	104	146	2020	100	320
30	110	152	3792	100	326
31	116	158	4247	100	332
32	123	165	3318	98	339
33	131	173	2646	100	347
34	138	180	3170	100	354
35	144	186	3638	95	360
36	151	193	2871	95	2
37	159	201	5084	100	10
38	165	207	5239	100	16
39	172	214	3332	85	23
40	187	229	3358	95	38
41	194	236	3592	95	45
42	201	244	3943	100	53
43	208	251	3063	100	60
44	215	257	3972	95	66
45	221	263	3381	100	72
46	229	271	3654	95	80

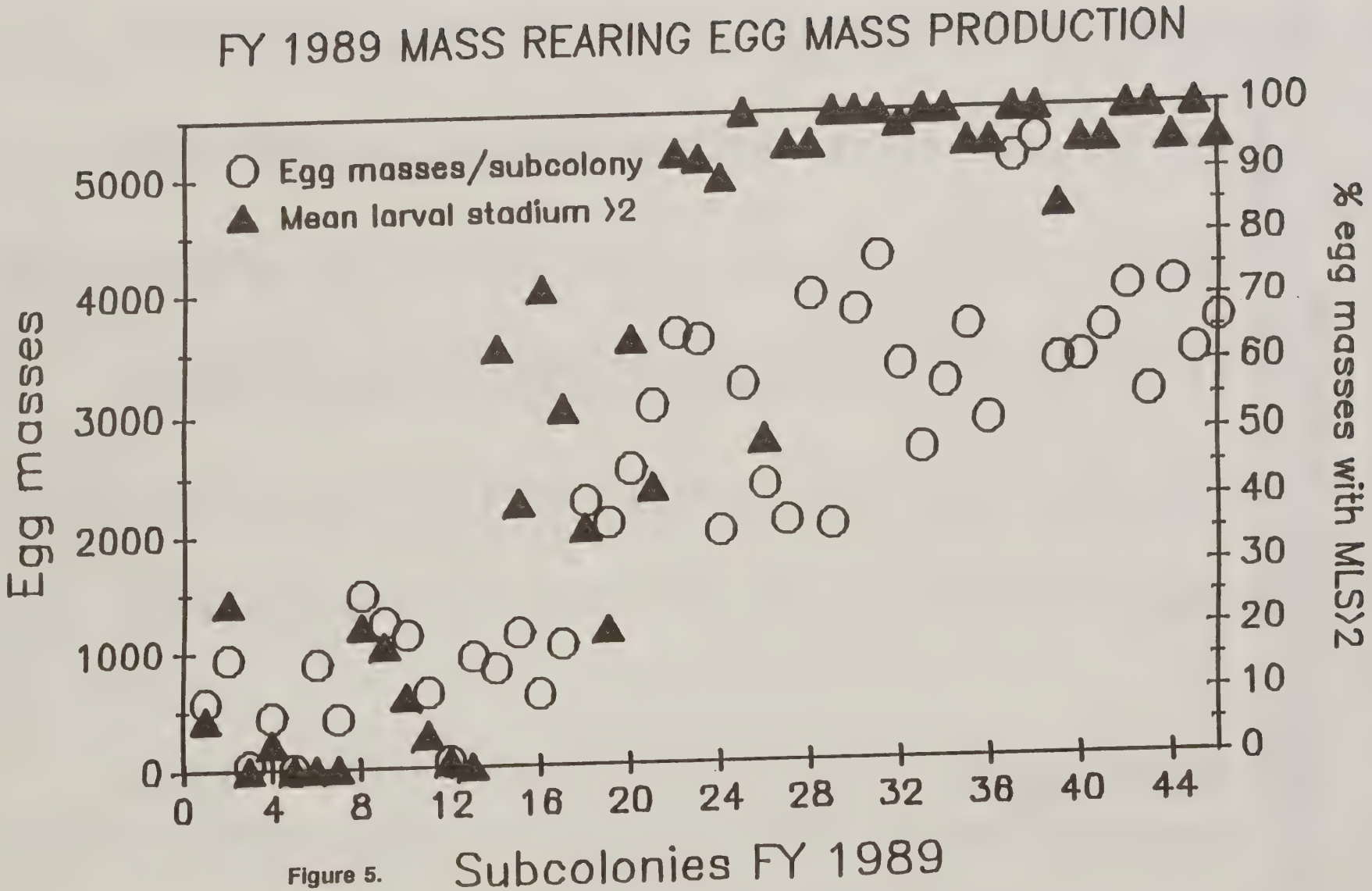

FY 1989 MASS REARING EGG MASS PRODUCTION
Egg masses/subcolony
Mean larval stadium >2
Egg masses
% egg masses with MLS>2
Subcolonies FY 1989
0
1000
2000
3000
4000
5000
0
10
20
30
40
50
60
70
80
90
100
0
4
8
12
16
20
24
28
32
36
40
44

Figure 5.

Project Number: GM 89.1.1.B
Project Title: 1989 Field Studies with Low Volume Applications of Dimilin 2F (Special) Against the Gypsy Moth (*Lymantria dispar* L.)
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leaders: W. McLane and J. Finney

Introduction

In 1990 approximately half of the total acreage (>1,000,000 acres) sprayed for gypsy moth control in the United States will be treated with Dimilin 25W. Nearly all Dimilin treatments will be applied at .03 lbs. AI/gallon/acre. The remaining acreage will be treated with various formulations of *Bacillus thuringiensis*. Most *Bt* is applied at one gallon per acre. However, a number of studies have demonstrated that the material can be applied successfully undiluted at 32 to 64 ounces per acre.

Undiluted ultra low volume (ULV) applications can save money and time. Much less ferrying time is needed and each load can cover more acres under more ideal spray conditions. No need for mixing equipment and crew is an additional savings.

In 1988 Dimilin 2F (Special) was tested at ultra low volume in Pennsylvania on 50-acre plots (McLane, Finney, Roland, Yendol and Bohne). The ultra low volume treatments were compared to a standard of Dimilin 25W at .03 lbs. AI/gallon/acre. Dr. Yendol and staff at Pennsylvania State University conducted a residue study in two of the treatment areas using fluorometer determinations.

Population reduction was superior in (ULV) applications of 64 and 32 ounces per acre to that of the standard Dimilin 25W at a gallon per acre. An application of 16 ounces per acre was equal to the standard. Dr. Yendol found nearly twice as much residue in the quart per acre treatment as in the standard Dimilin 25W at a gallon per acre (Yendol, McLane, Roland and Reardon).

Table 1. Percent population change based on pre- and post-spray egg mass counts.

Treatment	Pre-spray EM/acre	Post-spray EM/acre	Percent change
128 oz.	1,110	50	- 95.4
64 oz.	1,101	10	- 99.0
32 oz.	1,193	4	- 99.6
16 oz.	1,103	66	- 94
Control	894	5,459	+510.6

Table 2. An estimate of the Dimilin spray (micrograms/cm^2 AI) found on oak foliage when aerially applied at 128 and 32 fl. oz. per acre.

Rate	N	Tree section	Microgram/cm^2 Mean(a)	Log 10 Mean(b)	Error
128 fl. oz./A	500	both	0.00912	-2.04	0.04
	250	lower	0.00676	-2.17	0.06
	250	upper	0.01202	-1.92	0.05
32 fl. oz./A	500	both	0.01413	-1.85	0.04
	250	lower	0.01148	-1.94	0.07
	250	upper	0.01698	-1.77	0.07

(a) Calculated using the geometric mean method.
(b) Transformed from Log10 mean value.

Numerous laboratory and limited field studies indicate that Dimilin can be successfully applied at dosages lower than those presently used (McLane & Finney, and Herbaugh, McLane & Finney). With increasing concerns of the general public about the use of all pesticides, it is important that we use the smallest effective amount possible. Dimilin 25W is presently used at .03 lbs. AI/gallon/acre.

During 1989 field studies continued with (ULV) applications of Dimilin 2F (Special) as well as lower dosages of each formulation.

Table 3. Treatments for 1989 Dimilin (ULV) and low dosage studies.

Formulation	Dosage/rate	Acres treated
Dimilin 25W	.03 lbs.AI/128oz/acre	200
Dimilin 25W	.015lbs.AI/128oz/acre	200
Dimilin 2F (SP)	.03 lbs.AI/32oz/acre	200
Dimilin 2F (SP)	.015lbs.AI/32oz/acre	200
Dimilin 2F (SP)	.03 lbs.AI/16oz/acre	200
Dimilin 4F	.03 lbs.AI/128oz/acre	400
Control	--	200

Approximately 400 acres were also treated with a Dimilin 4F (flowable) formulation.

Methods and Techniques

Twenty-four woodland plots were established on state game lands in Elk and Jefferson Counties, Pennsylvania. Plots were square and 50 acres in size and located a minimum of 400 feet apart. Boundary lines were surveyed and marked with fluorescent orange tape and each corner tree was marked with double

fluorescent orange tape and a tag identifying corners and plot numbers. Plots were located so that there would be a maximum number of corners on or near roadways.

Treatment evaluation consisted of pre- and post-spray egg mass counts, egg hatchability tests, post-spray larvae counts under burlap, defoliation observations and residue work using wash-off and HPLC techniques.

Within the center 10 acres of each plot, 10 prism points were established, 5 points on 2 parallel lines. During March and early April, pre-spray egg mass numbers were recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 80> percent with little virus load.

Burlap was placed on 5 oak trees at random in the center 10 acres of one plot in each treatment. Three counts were made on the number of gypsy moth larvae under each band following treatment. After each reading, all larvae were removed from under the burlaps.

At peak defoliation time (early July), a survey was conducted from the ground. Total defoliation of all oak species was estimated at each prism point within each experimental plot.

Within 4 hours of treatment, foliage was collected in one plot each of the 128, 32 and 16 ounce treatments. Foliage was collected from the top, mid- and lower crown of 10 trees within each plot. From each tree and each location within the crown, foliage was collected at each cardinal direction.

Ten leaves were selected for HPLC work and 10 for wash-off (fluorometric determinations) work at Pennsylvania State University.

All foliage was shot out of the trees using a 12-gauge shotgun with 2.75 and 3 inch shells. This technique worked very well except for some black and blue shoulders.

Foliage was shipped to the USDA, APHIS National Monitoring Laboratory at Gulfport, Mississippi for HPLC analysis. Ms. J. Finney, assisted by Dr. Yendol's staff at Pennsylvania State University, did the wash-off (fluorometric determinations) analysis.

An APHIS Cessna Ag-truck aircraft was used to apply all treatments. Applications of 128 ounces per acre were made with 8004 flat fan nozzle tips with lesser amounts applied through 8002 nozzle tips. The aircraft sprayed a 75-foot swath at 120 mph, approximately 50 feet above the target foliage. Boom pressure was 40 psi. Screens of 50 mesh size were used in each nozzle and in the nurse tank.

The aircraft was calibrated over the first spray plot. Mixing was done in a nurse tank and then material was pumped into the aircraft. Additions to the Dimilin 2F (Special) formulation were added as a tank mix at the base of operations, DuBois Airport. A boom timer was used to aid calibration.

All applications were made in the morning hours between 6:00-12:00 AM starting 6/1 and ending 6/3. Temperatures ranged between 50° - 75°F. Application was terminated when winds exceeded 6 mph or temperatures reached 80°F or humidity dropped below 45%.

At time of treatment, the majority of larvae were mid to late 2nd instar. In general, foliage was expanded 40-60 percent.

Results

All treatments gave excellent gypsy moth population reduction based on egg mass counts. The (ULV) and low dosage treatments were as effective as the standard of Dimilin 25W. However, it was unfortunate that a population reduction also occurred in the untreated controls and therefore treatment and control comparisons are compromised.

We had problems mixing the emulsifiers and additives with the Dimilin 2F (Special) formulation in the field. Although we did use a considerable amount of emulsifier, the mix was not good. The formulation needs to be worked on to correct this problem.

Once the material was in the aircraft, it dispersed well and caused no blockage of nozzles or screens.

A significant amount of rainfall occurred during the 3-day period following treatments. However, this should have had no effect on the efficacy based on laboratory tests (McLane and Finney).

Foliage protection was excellent in all treatments and larvae numbers under burlap were greatly reduced in all treatment plots.

As was the case in our 1988 study (Yendol, McLane, Roland and Reardon), the HPLC analysis recovered nearly as much Dimilin 2F (Special) in the 32 ounce/acre treatment as Dimilin 25W in the 128 ounce/acre application. This again demonstrates the excessive evaporation taking place with the Dimilin 25W formulation. The Dimilin 2F (Special) at 16 ounces/acre had less material on foliage than the Dimilin 25W treatment at 128 ounces per acre.

Most Dimilin 25W was found high up in the tree (twice that found in the mid- or lower crown), whereas the Dimilin 2F (Special) at 16 and 32 ounces was very uniform in its deposit from top to lower crown. This was likely due to the finer droplets produced by the 8002 flat fan tips being able to penetrate the foliage canopy more efficiently. Most larger droplets produced by the 8004 flat fan tips stayed near the top of the tree crown.

The wash-off tests (fluorometer determinations) conducted at Pennsylvania State University also identify most of the Dimilin 25W at 128 ounces per acre as being high up in the tree and the Dimilin 2F (Special) as being more uniform throughout the tree.

Based on 1988 and this year's data, it is evident that (ULV) applications of Dimilin 2F (Special) can be just as effective as the standard formulation now being used at 128 ounces per acre. It is therefore time to conduct a pilot test with Dimilin 2F (Special) so that operational programs can use it in the near future. However, before this can happen, the formulation has to be improved so that mixing does not have to take place in the field. If this requires registration, it must be addressed as soon as possible.

The Dimilin 4F formulation performed very well based on efficacy, mixing and handling. This is a formulation that should be registered for use as the Dimilin 25W is presently used. It could also be used as a Special for (ULV) applications.

Again, as in 1975, low dosages were very effective. It is important that this work continue so that lower dosages can someday be used to ease the environmental concerns. If lower dosages are not available for use in the future, we may not have Dimilin to use at all.

References Cited

Herbaugh, L. L., McLane, W. H., Stacy, C. R. and J. A. Finney. 1975. Field evaluation of candidate insecticides for gypsy moth control. Otis Annual Laboratory Report, September 1975 - March 1976.

McLane, W. H. and J. A. Finney. 1988. Laboratory screening of candidate pesticides and microbials against the gypsy moth. Otis Annual Laboratory Report, October 1987 - September 1988.

McLane, W. H., Finney, J. A., Roland, T. J. and W. G. Yendol. 1988. Field studies with low volume applications of Dimilin. Otis Annual Laboratory Report, October 1987 - September 1988.

Yendol, W. G., McLane, W. H., Roland, T. J. and R. A. Reardon. 1988. A study to determine Dimilin residue on a northeastern oak forest using two spray mixers applied by air. Special Report to Uniroyal Chemical Company.

Table 4. Percent population change based on pre- and post-spray egg mass counts.

Formulation	Dosage/rate lbs.AI/oz/acre	Pre-spray[1/] Egg masses/acre	Post-spray[1/] Egg masses/acre	Percent change
Dimilin 25W	.03/128oz	635	14	- 97.7
Dimilin 25W	.015/128oz	544	12	- 97.8
Dimilin 2F(SP)	.03/32oz	801	3	- 99.1
Dimilin 2F(SP)	.015/32oz	1902	0	-100
Dimilin 2F(SP)	.03/16oz	705	1	- 99.8
Dimilin 4F	.03/128oz	946	11	- 98.8
Control		536[2/]	369[2/]	- 31.1

1/ Average of 4 spray plots
2/ Average of 5 untreated plots

Table 5. Average number of larvae under burlaps after spraying and percent defoliation at peak defoliation time.

Formulation	Dosage/rate lbs.AI/oz/acre	Larvae under burlap	Percent defoliation
Dimilin 25W	.03/128oz	1.5	0-10
Dimilin 25W	.015/128oz	0	0-10
Dimilin 2F(SP)	.03/32oz	.33	0-10
Dimilin 2F(SP)	.015/32oz	0	0-10
Dimilin 2F(SP)	.03/16oz	.66	0-10
Dimilin 4F	.03/128oz	--	0-10
Control	--	6.88	20-30

Table 6. Rainfall during and after application time.

Date	Inches rain
6/1	0.48
6/2	0.0
6/3	0.22
6/4	0.33
6/5	0.46
6/6	0.05
6/7	0.39
6/8	0.01
6/9	0.07
6/10	0.07

Table 7. Average ppm of Dimilin recovered from 10 trees at various canopy locations using HPLC.

Formulation	Dosage/rate lbs.AI/oz/acre	High	Medium	Low	Average 3 locations
Dimilin 25W	.03/128oz	2.047	1.092	0.931	1.356
Dimilin 2F (SP)	.03/32oz	2.3	2.01	1.829	2.046
Dimilin 2F (SP)	.03/16oz	.846	.838	1.045	.909
Control	--	<.5	<.5	<.5	<.5

Table 8. Average fluorescence of dye recovered from 10 trees at various canopy locations.

Formulation	Dosage/rate lbs.AI/oz/acre	High	Medium	Low	Average 3 locations
Dimilin 25W	.03/128oz	364	332	248	315
Dimilin 2F(SP)	.03/32oz	378	424	428	410
Dimilin 2F(SP)	.03/16oz	253	285	307	282
Control	--	91	113	110	105

Title: Determination of Dimilin® Residues in Vegetation Samples
Analyst: R. Reeves
Contributors: D. Ladner

Introduction

One hundred twenty (120) vegetation samples (pre-treatments and post-treatments) for the Gypsy Moth Program were received from the Otis Methods Development Center by the National Monitoring and Residue Analysis Laboratory in Gulfport, Mississippi, for analyses. The samples were analyzed for Dimilin® content.

Objective

To analyze vegetation samples for Dimilin® residues. These analyses were accomplished by high performance liquid chromatography (HPLC). Selected samples were confirmed by gas chromatography/mass spectrometry (GC/MS).

Methodology

NMRAL analyzed the vegetation samples in accordance with Processing Procedure PR0096, "Analysis of Diflubenzuron (Dimilin®) in Vegetation by Reversed Phase Higher Performance Liquid Chromatography", October 26, 1989.

Analyses for Dimilin® were accomplished on a Hewlett-Packard (HP) 1084B High Performance Liquid Chromatography using Beckman System Gold Programmable Solvent Module 126 and HP Model 79875A UV Detector and the following parameters:

a. Chromatographic column - Altrex Ultrasphere® ODS, 4.6 x250 mm, 5 um particle area

b. Mobile phase - Acetonitrile/water/1,4 Dioxane (45:45:10), flow 3.0 mL/min

c. Temperature - Ambient

d. Detector wavelength - 254 nM

Results (see enclosed table).

Summary

Of the one hundred twenty (120) vegetation samples analyzed, forty-eight (48) had residues with a high of 6.79 parts per million (ppm).

Residue Results of Dimilin in Vegetation Samples Received for the Gypsy Moth Program - Fiscal Year 1989

Lab Number	Site Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Dimilin
AA16969	CC0101	Luzerne, PA	Leaves	0	NA	06/02/89	<0.5
AA16970	CC0201	"	"	0	NA	06/02/89	<0.5
AA16971	CC0301	"	"	0	NA	06/02/89	<0.5
AA16972	CC0102	"	"	0	NA	06/02/89	<0.5
AA16973	CC0202	"	"	0	NA	06/02/89	<0.5
AA16974	CC0302	"	"	0	NA	06/02/89	<0.5
AA16975	CC0103	"	"	0	NA	06/02/89	<0.5
AA16976	CC0203	"	"	0	NA	06/02/89	<0.5
AA16977	CC0303	"	"	0	NA	06/02/89	<0.5
AA16978	CC0104	"	"	0	NA	06/02/89	<0.5
AA16979	CC0204	"	"	0	NA	06/02/89	<0.5
AA16980	CC0304	"	"	0	NA	06/02/89	<0.5
AA16981	CC0105	"	"	0	NA	06/02/89	<0.5
AA16982	CC0205	"	"	0	NA	06/02/89	<0.5
AA16983	CC0305	"	"	0	NA	06/02/89	<0.5
AA16984	CC0106	"	"	0	NA	06/02/89	<0.5
AA16985	CC0206	"	"	0	NA	06/02/89	<0.5
AA16986	CC0306	"	"	0	NA	06/02/89	<0.5
AA16987	CC0107	"	"	0	NA	06/02/89	<0.5
AA16988	CC0207	"	"	0	NA	06/02/89	<0.5
AA16989	CC0307	"	"	0	NA	06/02/89	<0.5
AA16990	CC0108	"	"	0	NA	06/02/89	<0.5
AA16991	CC0208	"	"	0	NA	06/02/89	<0.5
AA16992	CC0308	"	"	0	NA	06/02/89	<0.5
AA16993	CC0109	"	"	0	NA	06/02/89	<0.5
AA16994	CC0209	"	"	0	NA	06/02/89	<0.5
AA16995	CC0309	"	"	0	NA	06/02/89	<0.5
AA16996	CC0110	"	"	0	NA	06/02/89	<0.5
AA16997	CC0210	"	"	0	NA	06/02/89	<0.5
AA16998	CC0310	"	"	0	NA	06/02/89	<0.5

Lab Number	Site Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Dimilin
AA16999	100101	Luzerne, PA	Leaves	1	06/03/89	06/03/89	0.7
AA17000	100201	"	"	1	06/03/89	06/03/89	0.55
AA17001	100301	"	"	1	06/03/89	06/03/89	<0.5
AA17002	100102	"	"	1	06/03/89	06/03/89	<0.5
AA17003	100202	"	"	1	06/03/89	06/03/89	0.53
AA17004	100302	"	"	1	06/03/89	06/03/89	<0.5
AA17005	100103	"	"	1	06/03/89	06/03/89	1.15
AA17006	100203	"	"	1	06/03/89	06/03/89	<0.5
AA17007	100303	"	"	1	06/03/89	06/03/89	<0.5
AA17008	100104	"	"	1	06/03/89	06/03/89	<0.5
AA17009	100204	"	"	1	06/03/89	06/03/89	<0.5
AA17010	100304	"	"	1	06/03/89	06/03/89	0.622
AA17011	100105	"	"	1	06/03/89	06/03/89	<0.5
AA17012	100205	"	"	1	06/03/89	06/03/89	<0.5
AA17013	100305	"	"	1	06/03/89	06/03/89	3.01
AA17014	100106	"	"	1	06/03/89	06/03/89	0.70
AA17015	100206	"	"	1	06/03/89	06/03/89	<0.5
AA17016	100306	"	"	1	06/03/89	06/03/89	0.68
AA17017	100107	"	"	1	06/03/89	06/03/89	<0.5
AA17018	100207	"	"	1	06/03/89	06/03/89	<0.5
AA17019	100307	"	"	1	06/03/89	06/03/89	<0.5
AA17020	100108	"	"	1	06/03/89	06/03/89	<0.5
AA17021	100208	"	"	1	06/03/89	06/03/89	<0.5
AA17022	100308	"	"	1	06/03/89	06/03/89	<0.5
AA17023	100109	"	"	1	06/03/89	06/03/89	<0.5
AA17024	100209	"	"	1	06/03/89	06/03/89	2.99
AA17025	100309	"	"	1	06/03/89	06/03/89	3.19
AA17026	100110	"	"	1	06/03/89	06/03/89	<0.5
AA17027	100210	"	"	1	06/03/89	06/03/89	1.56
AA17028	100310	"	"	1	06/03/89	06/03/89	3.26
AA17029	200101	"	"	1	06/01/89	06/01/89	<0.5

Lab Number	Site Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Dimilin
AA17030	200201	Luzerne, PA	Leaves	1	06/01/89	06/01/89	<0.5
AA17031	200301	"	"	1	06/01/89	06/01/89	<0.5
AA17032	200102	"	"	1	06/03/89	06/01/89	3.32
AA17033	200202	"	"	1	06/01/89	06/01/89	2.68
AA17034	200302	"	"	1	06/01/89	06/01/89	<0.5
AA17035	200103	"	"	1	06/01/89	06/01/89	<0.5
AA17036	200203	"	"	1	06/01/89	06/01/89	<0.5
AA17037	200303	"	"	1	06/01/89	06/01/89	<0.5
AA17038	200104	"	"	1	06/01/89	06/01/89	6.14
AA17039	200204	"	"	1	06/01/89	06/01/89	<0.5
AA17040	200304	"	"	1	06/01/89	06/01/89	1.07
AA17041	200105	"	"	1	06/01/89	06/01/89	4.17
AA17042	200205	"	"	1	06/01/89	06/01/89	1.67
AA17043	200305	"	"	1	06/01/89	06/01/89	1.03
AA17044	200106	"	"	1	06/01/89	06/01/89	0.94
AA17045	200206	"	"	1	06/01/89	06/01/89	1.31
AA17046	200306	"	"	1	06/01/89	06/01/89	1.83
AA17047	200107	"	"	1	06/01/89	06/01/89	1.70
AA17048	200207	"	"	1	06/01/89	06/01/89	<0.5
AA17049	200307	"	"	1	06/01/89	06/01/89	1.21
AA17050	200108	"	"	1	06/01/89	06/01/89	1.69
AA17051	200208	"	"	1	06/01/89	06/01/89	2.23
AA17052	200308	"	"	1	06/01/89	06/01/89	1.67
AA17053	200109	"	"	1	06/01/89	06/01/89	0.98
AA17054	200209	"	"	1	06/01/89	06/01/89	<0.5
AA17055	200309	"	"	1	06/01/89	06/01/89	<0.5
AA17056	200110	"	"	1	06/01/89	06/01/89	<0.5
AA17057	200210	"	"	1	06/01/89	06/01/89	0.53
AA17058	200310	"	"	1	06/01/89	06/01/89	<0.5
AA17059	450101	"	"	1	06/03/89	06/03/89	1.31
AA17060	450201	"	"	1	06/03/89	06/03/89	<0.5

Lab Number	Site Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Dimilin
AA17061	450301	Luzerne, PA	Leaves	1	06/03/89	06/03/89	0.88
AA17062	450102	"	"	1	06/03/89	06/03/89	<0.5
AA17063	450202	"	"	1	06/03/89	06/03/89	0.61
AA17064	450302	"	"	1	06/03/89	06/03/89	<0.5
AA17065	450103	"	"	1	06/03/89	06/03/89	6.54
AA17066	450203	"	"	1	06/03/89	06/03/89	<0.5
AA17067	450303	"	"	1	06/03/89	06/03/89	5.43
AA17068	450104	"	"	1	06/03/89	06/03/89	4.62
AA17069	450204	"	"	1	06/03/89	06/03/89	4.11
AA17070	450304	"	"	1	06/03/89	06/03/89	1.88
AA17071	450105	"	"	1	06/03/89	06/03/89	<0.5
AA17072	450205	"	"	1	06/03/89	06/03/89	0.56
AA17073	450305	"	"	1	06/03/89	06/03/89	<0.5
AA17074	450106	"	"	1	06/03/89	06/03/89	<0.5
AA17075	450206	"	"	1	06/03/89	06/03/89	<0.5
AA17076	450306	"	"	1	06/03/89	06/03/89	1.16
AA17077	450107	"	"	1	06/03/89	06/03/89	<0.5
AA17078	450207	"	"	1	06/03/89	06/03/89	<0.5
AA17079	450307	"	"	1	06/03/89	06/03/89	2.46
AA17080	450108	"	"	1	06/03/89	06/03/89	<0.5
AA17081	450208	"	"	1	06/03/89	06/03/89	6.32
AA17082	450308	"	"	1	06/03/89	06/03/89	1.57
AA17083	450109	"	"	1	06/03/89	06/03/89	1.18
AA17084	450209	"	"	1	06/03/89	06/03/89	1.30
AA17085	450309	"	"	1	06/03/89	06/03/89	1.30
AA17086	450110	"	"	1	06/03/89	06/03/89	6.79
AA17087	450210	"	"	1	06/03/89	06/03/89	5.69
AA17088	450310	"	"	1	06/03/89	06/03/89	2.99

Lower limit of detection = 0.5 parts per million (ppm).

Project Number: GM 89.1.1.C
Project Title: 1989 Field Studies with High Dosage Applications of Foray 48B Against the Gypsy Moth (*Lymantria dispar* L.)
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leaders: W. McLane and J. Finney

Introduction

For the past seven years, *Bacillus thuringiensis* (Bt) has been used to treat an average of 49 percent of the total acreage sprayed for gypsy moth control in the United States (Gypsy Moth News, 1983-1989).

Dosages and rates of application with Bt have changed from year to year. Presently most applications are applied at 20 BIU/gallon/acre. However, a number of field tests and operational programs indicate good efficacy with undiluted applications. On the other hand, poor results have caused users to steadily increase dosages.

Dr. DuBois has tested a number of Bt formulations at high dosages and found that the dosage mortality curve becomes very flat with most after 20 BIU AI/acre (DuBois, 1989). He found Foray 48B, a Novo Laboratories Bt formulation, to continue to produce a steep curve at the higher dosages.

Laboratory studies at the USDA, APHIS, Otis Methods Development Center show Foray 48B to be a very effective Bt formulation. Otis studies also show longer field residual than the other Bt formulations (McLane, Finney and Darling).

This being true, it may be possible to treat isolated infestations with only a single or double application of Foray 48B at high dosage to achieve eradication. Presently three applications are used and are often followed by mass trapping.

In 1989, a total of 12 plots, 50 acres in size, were sprayed with high dosages of Foray 48B in north central Pennsylvania.

Foray 48B was applied at 20 BIU/gallon/acre using two applications 8 days apart. A diluted application of 40 BIU/gallon/acre was applied to 5 spray plots. This application was applied with two fly overs, 20 BIU/64 oz./acre each time. Each application was applied using a different flight path, giving a checkerboard effect. All plots were treated within a 4-hour period. Two plots were treated with undiluted Foray 48B at 24 BIU/46 oz./acre. Five untreated plots were used as a control.

Methods and Techniques

Seventeen woodland plots were established on state game lands in Elk and Jefferson Counties, Pennsylvania. Plots were square and 50 acres in size, and located a minimum of 400 feet apart. Boundary lines were surveyed and marked with fluorescent orange tape and each corner tree was marked with double fluorescent orange tape and a tag identifying corners and plot numbers. Plots were located so that there would be a maximum number of corners on or near roadways.

Treatment evaluation consisted on pre- and post-spray egg mass counts, egg hatchability tests, post-spray larvae counts under burlap, and defoliation observations.

Within the center 10 acres of each plot, 10 prism points were established, 5 points on 2 parallel lines. During March and early April, pre-spray egg mass numbers were recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 80> percent with little virus load.

Burlap was placed on 5 oak trees at random in the center 10 acres of one plot in each of two treatments and the control. Three counts were made on the number of gypsy moth larvae under each band following treatment. After each reading, all larvae were removed from under the burlaps.

At peak defoliation time (early July), a survey was conducted from the ground. Total defoliation of all oak species was estimated at each prism point within each experimental plot.

All treatments of Foray 48B were made with a USDA, APHIS, Cessna Ag-truck aircraft. The aircraft used a 75 foot swath at 120 mph, approximately 50 feet above the target foliage. Flat fan (8004-90° to slip stream) tips were used to apply the gallon per acre applications. They were also used for the undiluted application; however, they were pointed at 45° into the slip stream.

One gallon per acre applications were started with no nozzle screens. After 7 passes, the aircraft returned to the airport with 3 blocked nozzle tips. Nozzle screens of 50 mesh size were added to each nozzle and spraying continued. A 50 mesh in-line screen was used in the aircraft and a 50 mesh screen between the nurse tank and aircraft. After doing the first 5 plots, there was quite a build-up of solids from the Bt formulation on the wire mesh screens. Following treatment of the second series of 5 plots, there was some build-up of solids on the main screen in the aircraft and nurse tank. There were some solids on nozzle screens at each end of the boom. For the second treatment of the double application plots and the 2 plots treated with undiluted material, red slotted screens (Spray Systems 4514-NY 10 #2) were used. Both formulations (diluted and undiluted) passed through these screens with no build-up of material on screens or nozzle tips clogging.

The aircraft was calibrated over the first spray plot by use of plot size and boom timer.

Mixing was conducted in a nurse tank and then material was pumped into the aircraft using the overhead loading technique. There was a 50 mesh screen located on the outboard side of the nurse tank pump. The water used for the diluted formulations was from a local brook and had a pH of 6.2. For undiluted applications Foray 48B was loaded directly into the aircraft.

All treatments were conducted between May 28th and June 5th between 6:00 - 11:00 AM. Temperatures ranged between 36° and 66° with an average of 52°F. Relative humidity ranged from 50 to 92% with an average of 58%. At time of the first application at 20 BIU/gallon/acre, May 28th, little foliage was present on white oak. General foliage development was 30-40 percent with gypsy moth larvae averaging mid 2nd instar in size. White oak foliage was 20 percent and general foliage 50-60 percent expanded at time of last treatments. Most gypsy moth larvae were late 2nd instar at this time.

Pre-spray egg mass density ranged from 75 to 1,756 per acre with an average of 528.

Host trees were mainly red, white and chestnut oak. However, there was a greater amount than desirable of northern hard wood (cherry, maple and beech) in some of the plots).

Results

There was no significant differences between any of the treatments, with significant differences between the treatments and the untreated control. All treatments gave excellent population reduction and foliage protection. The Foray 48B treatments were as effective as a number of Dimilin 25W and Dimilin 2F (Special) treatments that were made in the same general area.

These excellent results should be viewed cautiously since a 31 percent population reduction occurred in the untreated control plots.

Burlaps in plots receiving two applications (8 days apart) at 20 BIU/gallon/acre had approximately half the number of larvae found in the control plots. Population reduction based on egg masses was excellent.

For all treatments foliage protection averaged 90-100 percent with the control at 70-80 percent.

The Foray 48B flowed well from the 55 gallon drums, mixed and handled well even with early morning temperatures near freezing.

When making undiluted applications, a build-up a material was observed around the nozzle tips. The external part of the nozzle tip should be checked and cleaned periodically.

Based on these results, single applications of Foray 48B at high dosages can be effective in causing significant population reduction. It may be possible to use single applications of Foray 48B at high dosages to eradicate isolated infestations. However, these types of dosages should be further tested in more suitable gypsy moth populations.

Table 1. Treatments with Foray 48B in 1989 field plots near Brockway, Pennsylvania.

Dosage/rate/acre	Number of applications	Nozzle size	Direction	Acres
20 BIU/128 oz.	2 (8 days apart)	8004	90°	250
40 BIU/128 oz.	1	8004	90°	250
24 BIU/64 oz.	1	8004	45°F	100
Control	--	--	--	250

Table 2. Average number of larvae under burlaps and percentage of defoliation in spray plots treated with Foray 48B.

Dosage/rate/acre	Number of applications	Ave. larvae under burlap	Defoliation
20 BIU/128 oz.	2 (8 days apart)	3.75	0-10
40 BIU/128 oz.	1	.14	0-10
24 BIU/64 oz.	1	--1/	0-10
Control	--	6.88	20-30

1/ No burlap for this treatment

Table 3. Population change in plots treated with Foray 48B and untreated controls based on pre- and post-spray gypsy moth egg mass counts.

Dosage/rate/acre	Number of applications	Pre 1/ EM/A	Post 1/ EM/A	Percent change
20 BIU/128 oz.	2 (8 days apart)	564	10	- 98.2
40 BIU/128 oz.	1	566	4	- 99.2
24 BIU/64 oz.	1	445	0	-100
Control	--	536	369	- 31

1/ Average number of egg masses for all replications of each treatment

References Cited

Gypsy Moth News, 1983-1989, USDA, Forest Service Numbers 7-20.

DuBois, N. R., Efficacy of *Bacillus thuringiensis* formulations against the gypsy moth. Proceedings of the 1988 National Gypsy Moth Review, Dearborn, Michigan, January 10-12, 1989.

McLane, W. H., J. A. Finney and R. R. Darling. 1988. Laboratory screening of candidate pesticides and microbials against the gypsy moth. Otis Annual Laboratory Report, October 1987 - September 1988.

Project Number: GM 89.2.1
Project Title: 1989 Disparlure dispenser formulation evaluations -- field bioassay.
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leader: V. Mastro, E. Paszek, C. P. Schwalbe
Cooperator: B. Leonhardt

During 1988 and 1989, a large effort was undertaken to find and evaluate pheromone dispensers which could be used in the gypsy moth survey and detection program. Results of field bioassaying candidate dispensers from six commercial manufacturers and two USDA-manufactured dispensers are described in the following narrative. Descriptions of all dispenser designs are presented in Table 1.

It is desirable in the gypsy moth survey and detection program to use pheromone dispensers that have a long field life. The gypsy moth flight period usually is 3-5 weeks in duration. In addition, because of manpower scheduling, traps are often placed in the field several weeks or in some cases months before flight begins. To determine the aging characteristics of candidate dispensers, lots of each type were pre-aged in a greenhouse for various lengths of time prior to testing. We planned to pre-age candidate dispensers for four lengths of time (4, 8, 12 and 16 weeks) prior to testing. Not all candidate dispensers, however, were received in time to age them for all periods.

Because of the large number of treatments involved in this comparison, a two-tiered approach was used for testing. In the first tier of tests, five plots were established, one for each group of dispensers aged for the five time periods (i.e., 0, 4, 8, 12 and 16 weeks). Within a plot, five blocks (lines) were established which would accommodate all treatments (e.g. randomized complete block design). Traps were checked and re-randomized three times. USDA milk carton traps spaced 50 meters apart were used for all testing. The objective of these initial comparisons was to identify "good" candidates which would be further tested in the second series.

In the second level of testing, six dispenser formulations aged for the five periods (6 dispensers X 5 ages = 30 treatments) were included in the same plot. A standard (100 μg of Plimmer-Schwarz (+) disparlure on a cotton wick) was also included. Again, traps were arrayed in a randomized complete block design (five blocks) but were check and re-randomized four times. USDA milk carton traps on a 50-meter spacing were also used in this trial.

Results and Discussion

Table 2 presents the results of the first level of testing. Although all of the data are included on one table and it is tempting to read across rows (aging periods), *only within-column comparisons should be made.* This is because plots with different aged wicks were in different locations with different male densities. Although a standard cotton wick (100 μg of PS disparlure) was not included in this test, the Hercon 1988 has been previously compared with it (see Table 4) and its performance can be used as a relative standard.

Within the comparison of unaged wicks (0 weeks), traps baited with the two Hercon dispenser formulations (1988 and 1989) captured significantly more males than any other dispenser type. Traps baited with two other types of formulations (Phero-tech's and DD VII's; two formulations of each) also captured large numbers of males. Although captures were significantly less in traps baited with these four dispenser types than those baited with the Hercon formulations, they captured, in most cases, significantly more males than the other formulations tested (Table 2).

In the comparisons with dispensers aged for 4 weeks, traps baited with a candidate dispenser (DD VII-79) captured the greatest numbers of males. However, mean capture of males in traps baited with this dispenser were not significantly greater than either of the Hercon dispenser formulations (1988 and 1989), one Phero-tech (5%) formulation or the similar DD VII-77 formulation. In addition, in comparison to the 1988 Hercon dispenser, traps baited with the TRECE-W.C. and the Sentry A did not catch significantly different numbers of males.

In the comparison of 8-week aged dispenser, traps baited with the newer Hercon (1989) formulation captured the greatest mean number of male moths. Capture in traps baited with this formulation, however, was not significantly greater than capture in traps baited with Hercon 1988 or DD VII-77 wicks. Relative to the 1988 Hercon formulation, capture in traps baited with the Phero-tech 5% and DD VII-79 were not significantly lower.

After 12 weeks of aging, 1989 Hercon-baited traps again caught the greatest mean numbers of male moths. However, capture in traps baited with the 1988 Hercon, DD VII-77 or DD VII-79 were not significantly lower. Also, although the mean capture males for two other formulations (Consep and AgriSense) were lower than the 1988 Hercon, they were not significantly different.

After 16 weeks of aging, traps baited with a candidate dispenser (DD VII-77) captured the highest mean number of males. This capture rate, however, was not significantly higher than traps baited with either the Hercon 1988 nor the Phero-tech 5% formulation. Relative to the Hercon 1988 formulation, the Phero-tech 2.5%, the Hercon 1989 and the DD VII-79 formulations all captured significantly fewer males.

The two candidate formulations that used rubber dispensers (TRECE R.S., Briese-R.St.) consistently resulted in uniformly low numbers of males captured. In previous trials with rubber type dispensers, similar poor results have been noted. The two candidate Sentry formulations captured lower numbers of males than the Hercon 1988 dispenser at all aging periods. Also, performance of these two formulations appears to be similar to each other. Chemical emission rate analysis will determine if the release rate is too high or low from these PVC cylindrical tube dispensers. The AgriSense dispenser, when unaged (0 weeks), resulted in very poor field performance; however, as it aged, its performance, relative to the 1988 Hercon and other dispensers, improved. Similarly, the Consep and TRECE-W.C. dispensers improved relatively with age. Again, emission and residual analysis may determine the underlying reasons for their performance and suggest remedies.

At the conclusion of the first tier of tests based on the results and availability for all aging periods, six formulations were compared in the second level of testing (Table 3). The unaged Hercon 1988 formulation produced the highest mean male capture. As these dispensers aged, capture appears to drop off gradually; however, not significantly until they were 16 weeks old. Performance of the Hercon 1989 dispenser was similar; however, the male capture rate drops off sooner (i.e., at 12 weeks) and continues to drop further at 16 weeks. Compared to the cotton wick standard, capture rates with the Hercon 1989 dispenser are significantly lower at both the 12 and 16 week aging periods.

A PVC-twine (DD VII-77) formulation at all ages captured fewer males than the cotton standard; however, only wicks aged 12 weeks captured significantly fewer males. The low male capture with the DD VII-77 formulation with wicks aged 12 weeks is unexplained in light of the relatively high capture with this wick formulation aged for 16 weeks. The other PVC-twine formulation (DD VII-79) apparently is releasing nearly in the right range of lure for wicks aged up to 4 weeks, then male capture is significantly lower than the standard for the last three aging periods. The Phero-tech 2.5% formulation performed as well as the cotton standard when aged up to 8 weeks, but capture for traps baited with wicks aged for the two longer periods was significantly lower.

Conclusions

The Phero-tech and the PVC twine dispenser formulations appear to offer possible alternatives for baiting gypsy moth survey traps; however, additional developmental work is needed before either is acceptable. Although chemical analysis will be more informative, it appears that a higher loading rate in the Phero-tech formulation (5% vs 2.5%) is necessary to achieve performance similar to the cotton dispenser. However, the 5% formulation performance diminishes significantly after 8 weeks of aging. Although this performance is similar to the Hercon 1989 formulation, the loading rate is 5 times higher (2.5 vs. 0.5 mg). Given that 250 grams are used nationally each year at a price of ca. $200/gram, the increase in program cost would be from $50,000 to $250,000.

Disturbingly, the performance of the Hercon 1989 formulation is significantly different than the Hercon 1988 formulation. A possible explanation is that the residual pool of disparlure is depleted after 8 weeks and

emission rates drop off. Chemical analysis of emission rates and residual analysis may determine how these formulations differ from each other. Theoretically, the Hercon formulation could be improved markedly by an increase in initial loading. The PVC twine (DD VII-79) formulation appears to come the closest to matching the performance of the standard dispenser over all aging periods. Why the capture at 12 weeks of aging was significantly lower and generally (not significantly) lower than the standard cotton remains to be determined. The two dispensers should be modified and re-tested in 1990.

Other dispensers which showed promise in the first tier of testing include the Consep, TRECE W.C. and the Sentry formulation. The AgriSense's performance relative to the Hercon 1988 with aging may make it a candidate if the chemical analysis can explain its behavior and the deficiencies. Several dispensers present problems for placement in traps. The TRECE-W.C. could not readily be fastened to the standard twist-tie or the USDA-built holder. Small net sacks were used for placement of this dispenser. The AgriSense dispenser was also very brittle and required small net sacks for placement.

Table 1. Description of pheromone dispensers used in field test - 1989.

Dispenser Codes	Lot Designation	Dispenser type	Nominal Loading Rate/ dispenser	Manufacturer
Hercon 1988	D0048 (4 X 25mm)	laminate	0.5 mg	Hercon Environmental Co.
Hercon 1989	D0139 (4 X 25mm)	laminate	0.5 mg	Hercon Environmental Co.
Phero-tech 5%	-- (5 X 3mm dia)	PVC cylinder	2.5 mg	Phero Tech Inc.
Phero-tech 2.5%	-- (5 X 3mm dia)	PVC cylinder	1.25 mg	Phero Tech Inc.
DD VII-77	-- (165 X 1.5 mm dia)	PVC coated twine	0.5 mg	USDA
DD VII-79	-- (60 X 1.5 mm dia)	PVC coated twine	0.5 mg	USDA
Consep	047B42391	membrane-covered, microporous reservoir	1.0 mg	Consep Membrane, Inc.
TRECE - R.S.	GM3107-2	red rubber septa	0.5 mg	TRECE, Inc.
TRECE - W.C.	GM3107-3	zeta lure (white capsule)	0.5 mg	TRECE, Inc.
AgriSense	-- (ca 25 X 25mm)	selibate	0.5 mg	AgriSense, Inc.
Sentry A	A (25 X 3mm dia)	PVC cylinder	0.5 mg	Sentry, Inc.
Sentry B	B (25 X 3mm dia)	PVC cylinder	0.5 mg	Sentry, Inc.
Briese R. St.	--	surgical tube rubber cap	0.5 mg	M. Briese

Table 2. Mean[1/] numbers of males captured per observations in USDA milk carton traps baited with various pheromone formulations 7/18 - 7/23, 1989, Jefferson County, PA.

Dispenser Type	Age of Dispenser in Weeks				
	0	4	8	12	16
Blank	0.1 g	0.8 e	1.1 h	0.1 d	0.2 d
Hercon 1988	87.8 a	105.6 abc	144.6 ab	128.5 a	80.5 ab
Hercon 1989	74.4 a	114.1 ab	157.2 a	144.7 ab	58.5 c
Phero-tech 5%	42.7 b	84.9 abcd	105.2 bcd	73.7 c	63.7 abc
Phero-tech 2.5%	33.5 bc	61.3 d	94.9 cde	69.1 bc	64.1 bc
DD VII-77	43.9 bc	111.9 ab	135.3 abc	110.0 abc	100.4 a
DD VII-79	40.6 b	135.0 a	104.9 bcd	100.3 abc	46.7 c
Consep	22.9 de	57.1 d	54.4 f	76.8 abc	--
TRECE - R.S.	16.7 de	6.0 e	9.6 g	6.8 d	--
TRECE - W.C.	26.8 cd	87.3 bcd	59.2 f	67.6 c	--
AgriSense	13.2 e	51.1 d	79.8 def	95.7 abc	--
Sentry A	19.1 de	63.5 cd	70.2 ef	--	--
Sentry B	24.5 cd	48.0 d	70.1 ef	--	--
Briese - R.St.	3.7 f	3.5 e	--	--	--

1/ Within a column, means followed by the same letter are not significantly different at the 5% level of significance according the Duncan's Multiple Range Test. For analysis, numbers of males captured (n) were transformed to $\sqrt{n + 0.5}$; actual means are present for clarity. Traps for testing were arrayed in a randomized complete block design (5 blocks). The error term used for mean separation was the error for the interaction of BLOCK X TREATMENT. Traps were checked and re-randomized three times (i.e., 5 blocks X 3 readings = 15 observations per treatment).

Table 3. 1989. Disparlure dispenser formulation run-off trial, conducted 7/23 - 7/27, 1989, Jefferson County, Pennsylvania. Mean[1/] numbers of males captured per observation

Dispenser Formulation	WEEKS AGED PRIOR TO TESTING				
	0	4	8	12	16
Phero-tech 5%	107.5 bcdefgh	129.4 abcdef	122.7 abcdefg	65.1 h	72.7 fgh
Phero-tech 2.5%	81.6 defgh	76.6 defgh	70.5 fgh	67.8 gh	70.9 fgh
DD VII-77	111.2 abcdefgh	130.6 abcde	124.7 abcdefg	83.4 defgh	135.1 abcde
DD VII-79	137.5 abcd	135.0 abcde	76.3 efgh	69.1 fgh	53.7 h
Hercon 1988	175.1 a	159.6 abc	124.0 abcdef	130.3 abcdef	97.5 cdefgh
Hercon 1989	161.0 ab	145.1 abc	102.4 bcdefgh	95.5 bcdefgh	58.7 h
Cotton 100μg	154.1 ab				
Blank	0.1 i				

1/ Means followed by the same letter are not significantly different at the 5% level according to Duncan Multiple Range test. Actual means are presented; for analysis data was transformed to $\sqrt{n + 0.5}$.

Table 4. Extracted from 1988 Detection Dispenser Study, mean numbers[1/2/] of males captured per day per trap (standard error in parentheses).

Dispenser type lure content	Dispenser age in weeks 0	4	8	16
1987 Hercon (D0048-500μg)	26.30 abc (3.00)	23.30 abcde (3.87)	23.65 abcde (3.75)	15.15 defgh (3.61)
Meyer-Schwarz Cotton - 100μg	21.80 abcde (3.07)			

1/ For analysis data that were transformed to x' = x + 0.5, actual means are presented. Data from blank traps were not used in the analysis.

2/ Means followed by the same letter are not significantly different at the 5% level according to Tukey's Standardized Range Test.

Project Number: GM 89.2.2
Project Title: Development of a reduced-diapause gypsy moth strain
Report Period: May 1, 1989 to September 30, 1989
Report Type: Interim
Project Leader: R.W. Hansen

Introduction

North American populations of the gypsy moth undergo obligate diapause-mediated dormancy in the overwintering egg stage. In laboratory rearing situations, this process is simulated by storing egg masses for 150-200 days at 5-7°C. Egg mass cold storage accounts for about two-thirds of gypsy moth generational time in artificial culture. This process greatly lengthens gypsy moth research efforts involving more than one generation. However, reducing the duration of cold storage (e.g. less than 120 days) considerably lengthens the number of days required for the initiation and completion of egg hatch, usually an unacceptable compromise.

Plentiful anecdotal evidence suggested that, under field conditions, some eggs hatch in late summer or early fall, obviously foregoing a winter diapause. Hoy (1977, 1978) demonstrated that such "nondiapause" characteristics may be readily selected for, and after eight generations she developed the diapause-free "Hoy strain". The Hoy strain is still propagated at several laboratories but apparently exhibited poor growth and development under the OMDC rearing scenario (V.C. Mastro, pers. comm.).

The objective of this project includes the development of a "reduced-diapause" gypsy moth strain that exhibits desirable rearing performance characteristics under OMDC conditions. Ultimately, this reduced-diapause strain would be available for experimental work, as appropriate.

Methods

The "nondiapause", hereafter referred to as ND, strain was initiated in 1987. In early spring, feral egg masses (P_1 generation) were collected in Rhode Island. Larvae from these egg masses were reared to pupation on oak foliage and artificial diet throughout the summer. In August, adult pairs were mated in an outdoor insectary and resulting egg masses collected about three weeks later. The egg masses (G_1 generation) were held outdoors and, in September, considerable atypical early hatch was observed (generally 10-20 L_1 per egg mass). These larvae were collected and reared, to pupation, on artificial diet in the laboratory, serving as the "founders" of the ND strain (300-400 L_1).

The ND strain has been maintained as a "typical" near-wild strain (i.e. 150-180 day egg cold storage) beginning with generation G_1. (Present methods for evaluating and propagating near-wild strains may be found in Report GM 89.3.3.) In May 1989, a group of egg masses was selected from the ND (G_4) egg mass "pool" to investigate egg hatch and subsequent larval performance after various lengths of egg cold storage. Ten egg masses were removed after 50, 70, 90, 110, 130, and 172 days chill (5°C).

After removal from cold storage, egg masses were individually held in Petri dishes at 27°C, >60% RH with a 14:10 (L:D) photoperiod. Masses were examined daily and the L_1 hatch recorded. For the 50, 70, 90, and 110 day chill treatments, newly-emerged first instars were reared to pupation in 200-ml (6 oz) plastic cups (10-12 L_1/cup) provided with 85 ml of artificial diet. Cups were examined after 12 days and the number of larvae in each stadium recorded. Pupae from the 50 and 70 day chill treatments were collected, sexed, weighed 24-48 hr after pupation, and examined for pupal abnormalities (see Report 89.3.5). Adults originating from those larvae that hatched after 50 or 70 days chill were mated individually or in small groups to provide egg masses (G_5) for future experiments.

Results

The following table summarizes egg hatch data for the various cold storage treatments. Figure 1 graphically illustrates hatch profiles.

Chill (days @ 5°C)	# L_1 hatched	#L_1 hatched/EM Mean (2 SE)	Hatch (days postchill) Started	Completed
50	276	27.6 (14.2)	12	31
70	2017	201.7 (86.6)	8	24
90	2539	253.9 (115.2)	7	19
110	2488	248.8 (76.9)	6	14
130	2970	297.0 (112.0)	6	12
172	1274	127.4 (64.7)	4	10

Egg counts and determination of embryonation rates have not been completed. Thus, "percent hatch" data are not yet available.

As expected, the initiation and duration of egg hatch decreased, and the number of eggs hatched per egg mass generally increased, with longer cold storage; particularly noticeable is the reduction in the number of days required to complete egg hatch. However, a small number of eggs (ca. 28/mass) did hatch after only 50 days chill.

Larval developmental data at 12 days after L_1 establishment are summarized below. Survival of young larvae was somewhat reduced following 50 and 70 days egg mass cold storage. Mortality among larvae hatching after 70 days includes losses attributable to gypsy moth NPV (ca. 2.4% of initial population); cups containing virus-killed cadavers at 12 days were discarded. Feral gypsy moths were reared concurrently in the same chamber; these insects suffered moderate NPV-induced mortality and thus "cross-contamination" was likely. There was no evidence for NPV contamination among other chill lengths.

Chill (days @ 5°C)	N (% survival)	Mean stadium	2SE	CV(%)
50	239 (86.6)	3.43	0.09	19.5
70	1732 (85.9)	3.30	0.03	17.6
90	2291 (90.2)	3.32	0.03	21.0
110	2324 (93.4)	3.53	0.02	16.0

Pupal data for larvae reared after 50 and 70 days of cold storage have not been completely analyzed; ca. 17% of these pupal records require computer entry and analysis. However, a summary of pupal data currently available is presented below. Data from ND pupae reared during near-wild strain evaluation experiments (see Report GM 89.3.3) is included; these insects originated from larvae hatched after 150-160 days cold storage.

Chill (days @ 5°C)	Sex	N	Pupal weight: Mean	2 SE
50	M	71	0.6537	0.0454
	F	152	1.8864	0.0959
70	M	533	0.6473	0.0309
	F	362	1.8047	0.0468
150+	M	82	0.6314	0.0417
	F	90	1.7761	0.1293

Pupal weight, serving as an indicator of pupal "quality" (and as a predictor of fecundity among females), is quite similar among insects hatched after only 50 or 70 days chill and those hatched after standard cold storage (150 days or more).

Discussion

Egg masses given short chill treatments (i.e. 50 or 70 days) apparently exhibited reduced hatch rates and prolonged durations of hatch (>14 days). However, larvae hatching after these reduced cold treatments exhibit survival rates, development rates, and pupal weights generally similar to those for larvae hatching after longer cold storage. Thus, the apparent "disruption" of diapause-mediated dormancy affected hatch but not the "quality" of larvae that did hatch.

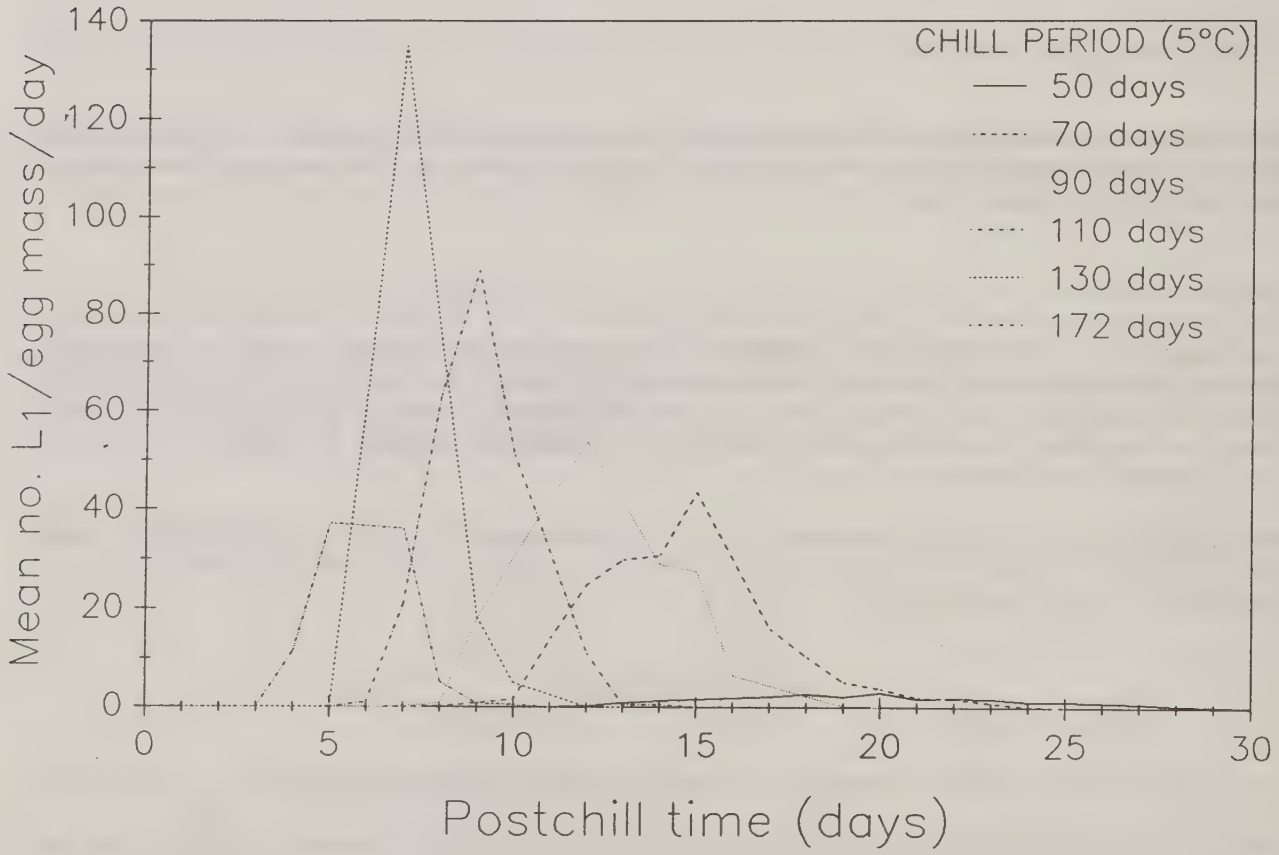
Progeny of the 50- and 70-day chill treatments (G_5) will be similarly evaluated after reduced lengths of cold storage. Continued selection, inbreeding, and evaluation of these lines will determine successful development of a "non-diapause" strain.

LITERATURE CITED

Hoy, M.A. 1977. Rapid response to selection for a nondiapausing gypsy moth. Science 196: 1462-1463.

Hoy, M.A. 1978. Selection for a non-diapausing gypsy moth: some biological attributes of a new laboratory strain. Ann. Entomol. Soc. Am. 71: 75-80.

FIGURE 1. Hatch profile of ND eggs (G_4) after varying durations of cold storage (n=10 egg masses per chill period).



Project Number: GM 89.2.3
Project Title: Investigations into Backcross Sterility
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leader: A. Pellegrini-Toole, V. Mastro, B. Ronberg

Although the F_1 Inherited Sterility technique has been successful in reducing or eliminating gypsy moth infestations, another method of producing sterile insects is being investigated. Based on the backcross sterility technique developed with *Heliothis virescens* (F.) and *H. subflexa* we began screening for backcross sterility with the gypsy moth, *Lymantria dispar* (L.), and some closely related lymantriad species. *Lymantria obfuscata* (Walker), the Indian gypsy moth, was the first candidate species imported for experimentation.

In August 1988, forty-nine *L. obfuscata* egg masses were received from Dr. R. L. Rajak, Plant Protection Advisor, New Delhi, India and placed in the Otis Methods Development Center quarantine facility. Thirty-nine were wild egg masses collected from Srinagar, India; the remaining ten were from a laboratory culture of insects maintained on foliage. The egg masses were examined for embryonation and parasites. Fifteen were found to have been parasitized by *Anastatus sp.*. The egg masses were held at room temperature approximately 22°C for 11 days, placed in an incubator and stepped down to 15°C for 11 days and then held at a constant 5°C with an ambient relative humidity of ca. 40%. Small groups of egg masses were removed from the incubator beginning 70 days after being placed at 5°C; some were held as long as 135 days.

Newly hatched larvae were infested onto standard gypsy moth artificial diet, 10 larvae/cup. The insects were reared at 25°C with a 14:10 light:dark photoperiod. Diet was changed as necessary. Larvae hatched from forty-five egg masses (mean number of larvae/egg mass was 59.73; S.D. = 48.52); in the remaining five, no hatch occurred. The mean larval development time for males was 37.07 days (S.D. 13.05, low of 22, high of 66) and for females it was 45.14 (S.D. 14.85, low of 29, high of 83). Gupta and Agarwal (1981) report a range of 55-69 days for males and 65-80.5 days for females under laboratory conditions.

Upon adult eclosion, moths were mated individually in 8 oz. paper cups. Pairs were made from adults originating from different egg masses to avoid inbreeding. A total of 306 "colony" matings were made. Eggs were held for at least 30 days at 25°C before refrigeration (7°C) to ensure embryonation.

Even though our major objective in the first generation was to colonize *L. obfuscata,* a small number of hybrid crosses were made. Fifty-four male *L. obfuscata* were mated with female *L. dispar* (Type 1) and seventeen male *L. dispar* were mated with female *L. obfuscata* (Type 2).

The resulting egg masses were hatched, and larvae reared in the same manner as described previously, and the resulting adults paired in the following ways:

Male Type	Female Type
Type 1 Hybrid	*L. dispar*
Type 1 Hybrid	*L. obfuscata*
Type 1 Hybrid	Type 1 Hybrid
L. dispar	Type 1 Hybrid
L. obfuscata	Type 1 Hybrid
Type 2 Hybrid	*L. dispar*
Type 2 Hybrid	*L. obfuscata*
Type 2 Hybrid	Type 2 Hybrid
L. dispar	Type 2 Hybrid
L. obfuscata	Type 2 Hybrid
Type 1 Hybrid	Type 2 Hybrid
Type 2 Hybrid	Type 1 Hybrid

Characteristics of the F_1 hybrid egg masses are presented in Table 1.

Table 1. Characteristics of hybrid egg masses.

Egg Mass Type	n	$\bar{X}$ # Eggs/E.M.	S.D.	n	$\bar{X}$ % Emb.	S.D.	n	$\bar{X}$ % Hatch	S.D.
Type 1	26	259.9	224.4	13	75.1	31.7	8	29.0	21.6
Type 2	8	98.4	130.3	7	37.0	27.4	4	60.5	26.1

Only one-half of the hybrid egg masses were retained for evaluation. Of the twenty-eight Type 1 matings, two did not produce an egg mass, thirteen out of twenty-six egg masses had no embryonation, and five out of thirteen embryonated egg masses had no hatch. Regarding the eight Type 2 matings, all produced an egg mass, one out of eight had no embryonation and three out of seven embryonated egg masses had no hatch.

The egg masses from the twelve backcross mating types will be ready for evaluation in early spring of 1990. The results of those matings will determine the future of *L. obfuscata* as a candidate in the backcross sterility program. Plans are underway to import two other species from Japan to continue the investigation.

Project Number:	GM 89.2.5
Project Title:	Disparlure Dispenser Loading versus Male Response in Various Gypsy Moth Population Densities
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leaders:	V. Mastro, E. Paszek, W. McLane, C. Schwalbe
Project Cooperator:	B. Leonhardt

Introduction

Earlier dose:response studies with (+) disparlure have been carried out using a variety of trap designs and in various population densities and therefore the dose:response relationship as described is a composite of many tests' results. Most of these studies were conducted using 6mm slices of cotton dental rolls (13mm dia.) as pheromone dispensers. A cotton dental roll dispenser loaded with 100μg of (+) disparlure has been adopted as a reference dose when testing new dispenser formulations (Schwalbe 1981). Also, the dental roll dispenser is used when bioassaying new lots of neat (+) disparlure. Emission rates from cotton dispensers have been described by Leonhardt et al (1987 and 1990). These emission rates are very rapid, necessitating replacement of dispensers on a 3-day interval in field studies.

Recently there has been a ongoing research effort to develop a trap for monitoring gypsy moth populations in the generally infested area. The trap currently in use (USDA milk carton) and pheromone dispenser (Hercon-laminate containing 500μg (+) disparlure) are unsuitable because traps rapidly reach capacity (~800 males) in dense gypsy moth populations. Currently the focus of the development effort is concentrated on reducing trap efficiency by reducing the (+) disparlure emission rate from the dispenser. Although this method shows promise, it is unknown if a low emission rate lure-baited trap performs similarly in all population densities. Possible variables affecting trap performance include different background levels of disparlure (emission from females) and changes in male behavior either related to or independent of female density.

The objectives of this study were to:

1) Describe the dose (emission rate) versus response (male capture) relationship of (+) disparlure.

2) Determine if and how the relationship changes with differences in native insect density.

3) Develop a research dispenser for disparlure that can easily be made in the laboratory, has a uniform emission rate and long field life. Such dispensers would provide a tool for bioassaying prospective lots of neat disparlure and serve as a standard when evaluating new production lots of dispensers.

Methods and Material

Dispensers were prepared by mixing neat (+) disparlure with PVC (S194-A Sinclair & Rush, St. Louis, MO) at the highest concentration 10mg/dispenser (1 gram). From this mixture, dilutions with PVC were made to provide a log dose series of concentrations ranging from 0.001 μg to 10,000μg/1 gram of PVC. One gram quantities of the mixtures were dispensed into porcelain spot plates and "cured" by holding in a 170°C oven for 10 minutes.

Emission rate evaluation of three of these dispenser loadings (10,000μg, 1,000μg and 100μg) were determined by the methods described by Leonhardt et al (1979, 1987).

Table 1. Emission rates from PVC dispensers held in an oven at 35°C with an airflow of 100ml/min.

Dispenser	ng/hr
PVC 10,000μg	242
PVC 1,000μg	22.2
PVC 100μg	5.7*
Cotton 100μg	470

* Calculated from inflection in the baseline of chromatograph.

For field testing, we attempted to select three sites which would provide a wide range of gypsy moth densities. In the sites we selected as "low" and "moderate" densities, four complete blocks (replicates) were established and they were read and randomized daily four and three times respectively. In the "high" density site, six complete blocks were established, and read and randomized five times. In the high density site, traps were read and randomized at intervals of 2-4 days. The high density site also differed in that it did not contain the two treatments with the highest dispenser loading rates (i.e., 1,000 and 10,000μg dispensers). Dispensers for all three sites were prepared and placed in the field fresh (unaged). A separate plot was established in the high density site to determine the aging characteristics of PVC dispensers and their expected field life. This plot was similar to the field plot which used unaged dispensers with the exception that all dispensers had been aged in a greenhouse for 9 weeks prior to testing.

USDA high capacity milk carton traps were used for all treatments at all sites. Trap spacing was held constant (50m) and traps were hung from limbs of trees between 1 - 1.5m in height.

Results and Discussion

Increased dispenser loading rates produced increasing male captures at all sites (Table 2). Traps baited with the two lowest dispenser loadings failed to consistently capture males in the light and moderate population density sites; however, they did capture more consistently in the high insect density site. Regression analysis was used to fit models to the data from three sites. A log-log transformation (Log_{10} males captured = b_0 + [b_1 x Log_{10} dispenser dose]) provided the best fit for the data (Figure 1). Slopes and intercepts of the three regression lines were compared. As one would expect, the intercepts of the three regression lines were significantly different ($P = .05$). Also, when the slopes of the lines were tested, they were all significantly different from each other. The steepest slope of the relationships was found in the model for the high density site (0.7072 x Log_{10} dose) and the slope decreased with decreasing insect density.

Table 2. Influence of disparlure dispenser loading on trap catch in gypsy moth populations of various male densities

Dispenser Dose (μg)	Mean Number of Males Captured per Trap per Day Population Densities		
	Low Jefferson Co PA	Moderate Jefferson Co PA	High Beltsville MD
0.001	0.00 (16)	0.08 (12)	2.4 (24)
0.01	0.00 (16)	0.33 (12)	4.3 (21)
0.1	0.13 (16)	0.25 (12)	8.3 (30)
1.0	0.31 (16)	1.50 (12)	11.3 (25)
10.0	1.38 (16)	13.50 (12)	69.0 (30)
100.0	4.25 (16)	26.58 (12)	66.3 (30)
1000.0	6.56 (16)	33.17 (12)	--
10000.0	11.13 (16)	47.5 (12)	--

$Log_{10} = B_0 + (B_1 \times [Log_{10} \text{ dose}])$
Low density = 0.010474 + 0.235025 (Log_{10} dose)
Mod density = 0.203018 + 0.405140 (Log_{10} dose)
High density = 0.925900 + 0.707215 (Log_{10} dose)

One interpretation of these data is that a given dispenser loading performs differently in various gypsy moth populations. Possibly high dispenser loading rates capture a larger percentage of the male population in high density sites than low density sites. Explanations include changes in male behavior in various densities which increase a male's threshold for response to pheromone, and/or increased male movement in high density sites which would expose more males to a trap's active zone. In low male density sites, the slope of the response may simply be depressed because the more efficient traps' "high dispenser loadings" deplete the local male population. Local depletion of males in low density sites with highly efficient traps would push the regression line towards horizontal if male movement into the trap's "active" zone is small or non-existent.

In all three sites, we were unable to determine the point at which the dispenser loading rate produced maximum male capture and then either declined or remained relatively constant. Measured emission rate for the highest dispenser loading was 242ng/hr. Earlier studies have shown that the reference cotton dispenser emits 470ng (+) disparlure/hour (Leonhardt et al 1990). Determining the dispenser loading which produces peak capture would provide a standard or reference dose for future comparisons of lots of neat (+) disparlure or new dispenser designs.

In the high gypsy moth density site, the aged dispensers performed similar to unaged dispensers (Table 3).

Table 3. Comparison of male capture in traps baited with unaged and aged[1/] PVC dispenser, Maryland 1989

Dispenser Type load (µg)	unaged			aged (9 weeks)		
	n	mean	S.E.	n	mean	S.E.
0.001	29	6.3	1.601	33	6.5	1.138
0.01	27	11.3	3.284	36	3.8	0.678
0.1	36	26.2	6.062	36	9.5	1.751
1.0	31	36.7	6.667	36	67.5	10.629
10	36	210.5	42.029	36	238.3	36.061
100	36	257.4	33.586	36	329.0	43.540

When regression models were fit to the data, the equations for the aged and unaged dispensers were respectively: $\log_{10}$ males = 0.801308 x 0.888075 ($\log_{10}$ dose) and $\log_{10}$ males = 0.9259 + 0.707215 ($\log_{10}$ dose). The two models are not significantly different (P = 0.5). This indicates that PVC dispensers have a field life of greater than nine weeks (i.e., greenhouse aging is accelerated because of higher temperatures; Leonhardt et al 1990). Laboratory emission rate data of aged dispensers now being completed will determine how emission rates vary with aging.

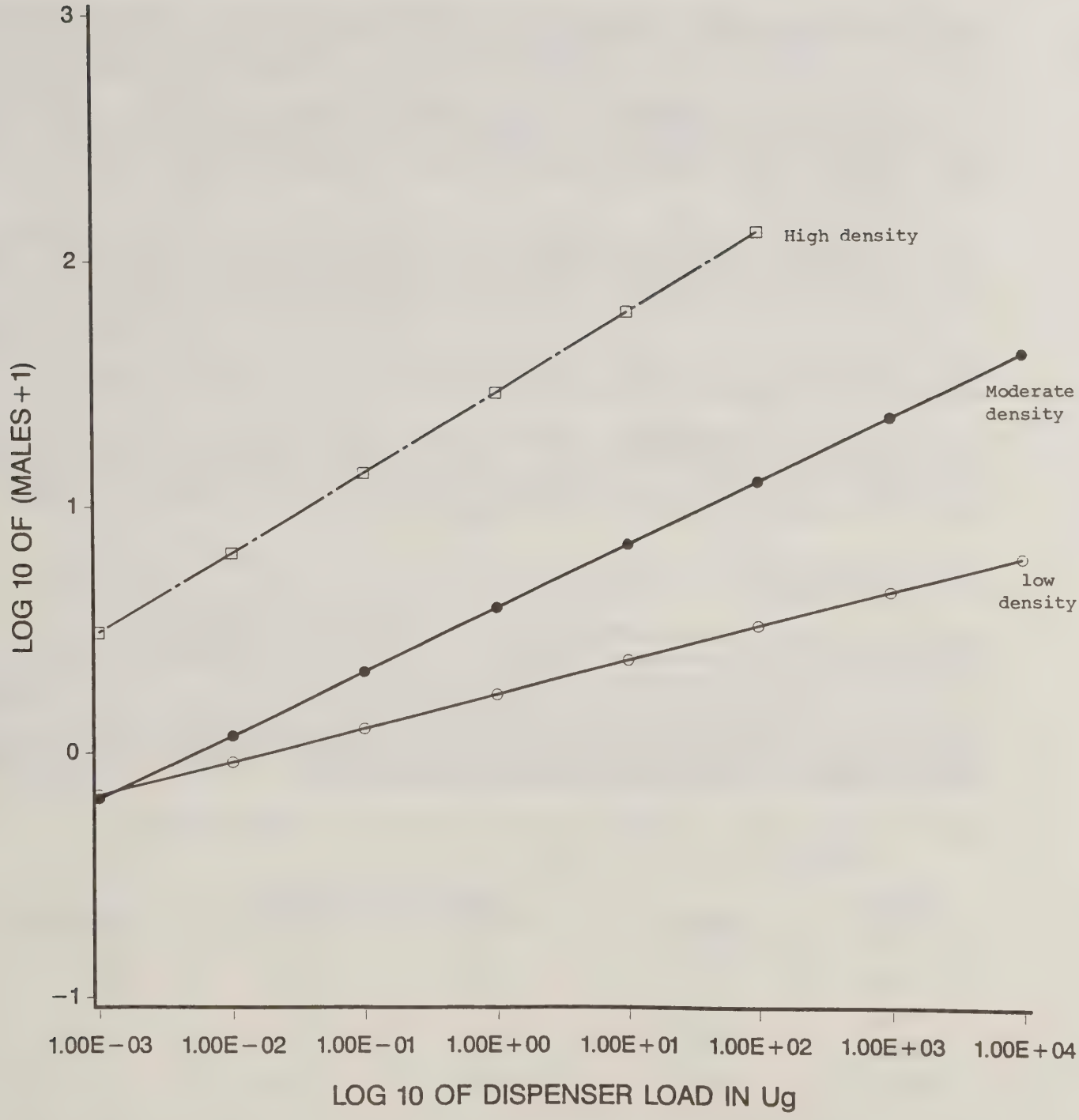
In summary, PVC dispensers with minor changes in the formulation appear to offer a good research tool for bioassaying lots of neat (+) disparlure and for use as a standard against which to compare new dispenser lots. Good characteristics include ease of laboratory preparation and long field life. Increased loading produces increased male capture and presumably increased emission rates.

With minor changes, it appears that this formulation is suitable for establishing a dose (emission rate) response (male capture) relationship for (+) disparlure. The preliminary data previously described suggest that this relationship changes with changes in population density; however, further study is required to determine if this is the case.

Additionally, the PVC dispenser, when loaded with small quantities (i.e., 1µg - 10µg) of (+) disparlure may provide the emission rates suitable for monitoring densities with traps in established gypsy moth populations.

Figure 1.

PHEROMONE DISPENSER LOAD VS MALE RESPONSE



Project Number: GM 89.2.6
Project Title: Evaluation of lots of neat (+) disparlure - 1989
Report Period: October 1, 1988 - September 30, 1989
Report Type: Interim
Project Leaders: V. Mastro, E. Paszek, W. McLane, C. P. Schwalbe
Cooperator: B. Leonhardt

Enantiomeric purity of disparlure (cis-7,8-epoxy-2-methyloctadecane) is critical when formulating dispensers for survey traps. Although purity of the configurational isomers can be quantified analytically, the most sensitive method of determining enantomeric purity of the attractive isomer, (+) is through bioassay. In 1989, an effort was made to identify new commercial sources of supply for (+) disparlure. A field bioassay and analytical analysis were used to compare samples from three prospective suppliers. Results of this comparison will be used for selecting which source of (+) disparlure will be used for supplying the 1990 national trapping program, and also for supplying a "strategic" stockpile in the event of future disruptions in the pheromone industry.

In all, three potential suppliers (Hercon [Nitto Denko], Andrulis, Orysenex) provided samples for testing. Although one supplier (Orysenex) later withdrew their offer, all three samples were field tested. A field bioassay was carried out in a light gypsy moth infestation in Jefferson County, Pennsylvania, between July 20 and July 26. Each of four lots of (+) disparlure (3 candidates and Plimmer-Schwarz control) was tested at 4 dispenser loading rates (1, 10, 100, 1000μg). Cotton dental rolls (6mm slices of 13mm diameter) were used as dispensers for all treatments. Dispensers were loaded the morning the test was initiated and replaced with freshly loaded dispensers after 3 days.

Standard USDA high capacity "milk carton" traps were used for all treatments. For placement, traps were hung from limbs of trees at between 1 - 1.5 m. in height. A randomized complete block (5 blocks) was used for trap layout with a fifty-meter spacing between traps and blocks (lines). Traps were read and re-randomized four times (= 20 observations/treatment).

Results and Discussion

Generally at the lowest dispenser loading rates, all materials performed similarly (Table 1). Trap catch for all but the Orysenex sample increased with loading rate. This lack of response with increasing dose is characteristic of dispensers loaded with a racemic mixture of the optical isomers. The material submitted by Hercon generally performed superior to all other materials tested, including the control. At the two highest dispenser loading rates, traps baited with the Hercon lot captured significantly more males than any material. Based on these results, we recommend that the (+) disparlure lots of choice for the 1990 program and for a strategic stockpile should be the product supplied by Hercon.

Table 1. Mean[1/] numbers of males captured per observation[2/] in USDA milk carton traps baited with various lots of (+) disparlure

Dispenser loading	Source of (+) disparlure			
	Hercon	Andrulis	Orysenex	P-S Control
1 μg	7.4 f	19.7 ef	8.0 f	13.1 f
10 μg	11.4 ef	13.2 ef	5.5 f	21.3 e
100 μg	91.4 b	41.9 d	5.6 f	57.9 cd
1,000 μg	121.7 a	43.5 d	5.8 f	62.7 c

1/ Means followed by the same letter are not significantly different at the 5% level, according to Duncan's Multiple Range test. For analysis, data were transformed to $\sqrt{\text{males} + 0.5}$. Actual means are presented for clarity.
2/ n = 20

Project Number:	GM 89.3.1
Project Title:	Gypsy Moth Forest Service Reimbursable Agreements - Mass Rearing
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leader:	G. Bernon

In FY89, the Mass Rearing Unit produced gypsy moths under three reimbursable agreements with the Forest Service: (1) Gypsy Moth Nucleopolyhedrosis Virus Production (NPV); (2) 8K F_1 Sterile Male Production; and (3) 8K Sterile Pupae Production.

1. **NPV Production:** The agreement was to produce 3000 acre equivalents of NPV (Gypchek). Otis mass-reared gypsy moth larvae were inoculated and sent to the Forest Service as frozen cadavers to be processed. The original agreement was to produce 1,500,000 larvae, assuming 500 larvae per acre equivalent of Gypchek. A production regime as outlined in Figure 1 was followed. Production started on November 14, 1988 and was terminated on April 12, 1989. A summary of the 69 production days is listed in Figure 2. An estimated 1,000,000 larvae were produced. The agreement was terminated early to start the Forest Service 8K Sterile Male Agreement (see Point 2 below). Consequently, the remaining production (500,000 larvae or 1,000 acre equivalents), was carried over to FY90 to be completed.

2. **8K Sterile Male Production:** The agreement was to produce 150,000 egg masses produced from sterile male parents for release in 1990. The 46 production days are summarized in Figure 3. This agreement was completed on time with an estimated 160,244 egg masses produced. This estimate was based on the assumption that only 80% of mated pairs produced egg masses and only 75% of those egg masses developed viable eggs. Therefore, actually 246,674 mated pairs were produced as shown in Figure 3.

3. **8K Sterile Male Pupae Production:** The agreement was to produce 100,000 sterile male pupae. This agreement was also completed on time as summarized in Figure 4. The pupae were ordered on 32 separate days; orders were met or exceeded on 19 days, with a total of 114,511 pupae produced (see Figure 4).

Summary

Although a limited number of gypsy moths were produced for Otis projects, the main thrust in FY89 was mass rearing for the Forest Service. In FY90 the Forest Service NPV Agreement will be expanded to a year-round project.

GYPSY MOTH - NPV PRODUCTION

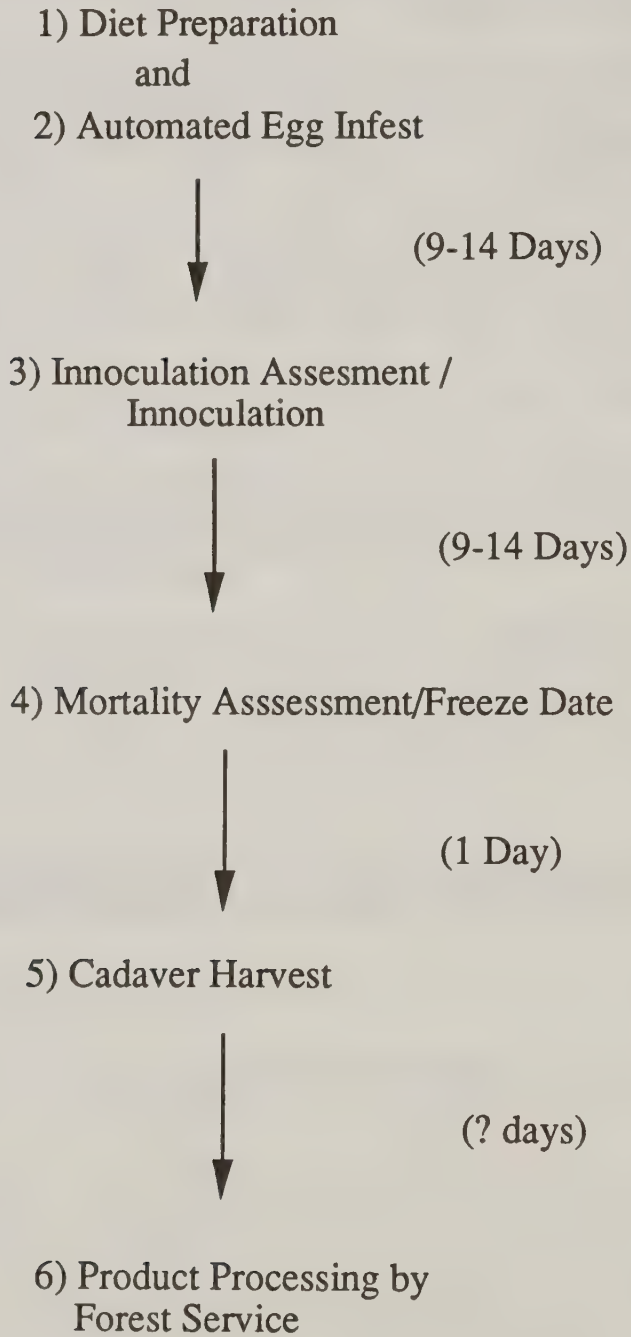


Figure 1.

Figure 2. FY89 Virus Collection Data

		VID[1]	# Cups
November	14	319	1890
	15	320	1890
	16	321	1890
	17	322	1890
	18	323	1890
	21	326	1890
	22	327	1890
	23	328	1890
	25	330	1890
	29	334	1890
	30	335	1890
December	1	336	1890
	2	337	1890
	5	340	1890
	6	341	1890
	7	342	1890
	8	343	1890
	9	344	1890
January	4	4	2520
	5	5	2520
	6	6	2520
	10	10	2520
	11	11	2520
	12	12	2520
	13	13	2520
	17	17	2520
	18	18	1260
	19	19	1260
	20	20	1260
	23	23	1890
	24	24	1260
	25	25	1260
	27	27	1410
	31	31	1260
February	1	32	1260
	2	33	1260
	3	34	1260
	8	39	1260
	9	40	1260
	14	45	1260
	15	46	1260
	16	47	1260
	17	48	1260
	22	53	1260
	23	54	1260
	24	55	1260
March	1	60	1260
	2	61	1260
	3	62	1260
	7	66	1260
	8	67	1260
	9	38	1260
	14	73	1260
	15	74	1260
	16	75	1260
	17	76	1260
	21	80	1260
	22	81	1260
	23	82	1260
	24	83	1260
	29	89	1260
	30	89	1260
	31	90	1260
April	5	95	1260
	6	96	1260
	7	97	1260
	11	101	1260
	12	102	1260
Total Number of Cups =			109140

Assume: Mean # Infected Larvae/Cup =	Total # Infected Larvae =
10	1091400[2]
9	982260
8	873120
7	763980
6	654840

[1] VID = Viral Infest Date
[2] Production mean was 10 larvae/cup

Figure 3. NPV Production

03-Nov-89
F_1 Production FY89

Average # Females per cup = 2.98

Infest Date	Harvest Date	F.S. Females Harvested	Males Harvested	Mating Pairs	# Cups	Extra Females Added	Number of Viable Egg Masses this Represents Date	Number of Viable Egg Masses this Represents Total	Predicted Date	Predicted Total	Deficit Date	Deficit Total
192	227	3500	5400	3500	1260		2100	2100	2646	2646	-546	-546
193	228	3100	4800	3100	1260		1860	3960	2646	5292	-786	-1332
194	229	3300	5000	3300	1260		1980	5940	2646	7938	-666	-1998
195	230	3500	5569	3500	1260		2100	8040	2646	10584	-546	-2544
198	233	4600	7300	4600	1260		2760	10800	2646	13230	114	-2430
199	234	6800	9700	6800	2520		4080	14880	5292	18522	-1212	-3642
200	235	5600	8100	5600	2520		3360	18240	5292	23814	-1932	-5574
201	236	3700	5700	3700	2520		2220	20460	5292	29106	-3072	-8646
202	237	4600	6500	4600	2520		2760	23220	5292	34398	-2532	-11178
205	240	4500	4900	4500	1260		2700	25920	2646	37044	54	-11124
206	241	2874	3500	2874	2520		1724	27644	5292	42336	-3568	-14692
207	242	2600	5100	2600	2520		1560	29204	5292	47628	-3732	-18424
208	243	3700	3600	3700	2520		2220	31424	5292	52920	-3072	-21496
209	244	3400	6000	7400	2520	4000	4440	35864	5292	58212	-852	-22348
212	248	4200	6200	6200	1260	2000	3720	39584	2646	60858	1074	-21274
213	249	4800	7400	5300	1260	500	3180	42764	2646	63504	534	-20740
214	250	1300	5600	1800	2520	500	1080	43844	5292	68796	-4212	-24952
215	251	7200	8700	7200	2518		4320	48164	5287.8	74083.8	-968	-25919
219	255	4500	6300	4900	2490	400	2940	51104	5229	79312.8	-2289	-28208
220	256	5100	8300	6500	2560	1400	3900	55004	5376	84688.8	-1476	-29684
221	257	9700	1070	9700	2370		5820	60824	4977	89665.8	846	-28841
222	258	14300	16300	15300	3660	1000	9180	70004	7686	97351.8	1494	-27347
223	261	4600	5100	4600	1260		2760	72764	2646	99997.8	114	-27233
226	261	4500	8900	4500	1260		2700	75464	2646	102643.8	54	-27179
227	262	9100	10400	9100	2520		5460	80924	5292	107935.8	168	-27011
228	263	9000	9900	9000	2520		5400	86324	5292	113227.8	108	-26903
229	264	9600	10800	9600	2520		5760	92084	5292	118519.8	468	-26435
230	265	9500	13300	9500	2520		5700	97784	5292	123811.8	408	-26027
233	268	4100	9200	4100	1260		2460	100244	2646	126457.8	-186	-26213
234	269	9500	10300	9500	2520		5700	105944	5292	131749.8	408	-25805
235	270	10200	13400	10200	2520		6120	112064	5292	137041.8	828	-24977
236	271	10200	14500	10200	2520		6120	118184	5292	142333.8	828	-24149
237	272	8500	13500	8500	2520		5100	123284	5292	147625.8	-192	-24341
240	275	4100	5100	4100	1260		2460	125744	2646	150271.8	-186	-24527
241	276	2500	3777	2500	1140		1500	127244	2394	152665.8	-894	-25421
242	277	4900	6100	4900	1260		2940	130184	2646	155311.8	294	-25127
243	278	5300	6700	5300	1260		3180	133364	2646	157957.8	534	-24593
244	279	4100	6000	4100	1110		2460	135824	2331	160288.8	129	-24464
249	284	4500	6200	4500	1260		2700	138524	2646	162934.8	54	-24410
250	285	5200	7400	5200	1260		3120	141644	2646	165580.8	474	-23936
251	286	5200	6700	5200	1260		3000	144644	2646	168226.8	354	-23582
254	289	5600	8300	5600	1260		3360	148004	2646	170872.8	714	-22868
255	290	5200	6900	5200	1260		3120	151124	2646	173518.8	474	-22394
256	291	5200	6700	5200	1260		3120	154244	2646	176164.8	474	-21920
257	292	5400	6384	5400	1260		3240	157484	2646	178810.8	594	-21326
258	293	4600	5700	4600	1260		2760	160244	2646	181456.8	114	-21212
	Total =	236874	317416	246674	81368							

Figure 4. 8K Production in 1989 - Sterile Pupae

Harvest Date	Infest Date	Males	Males Ordered	Difference
170	135	1526	1000	526
171	136	1902	1000	902
172	137	263	1000	-737
173	138	1743	1000	743
174	139	2264	2000	264
177	142	2861	2000	861
178	143	1913	1000	913
179	144	1934	1000	934
180	145	1621	1000	621
181	146	4284	4000	284
184	149	3142	4000	-858
185	150	2670	2000	670
186	151	2827	2000	827
187	152	3177	2000	1177
188	153	6458	6500	-42
191	156	7653	9000	-1347
192	157	2815	4500	-1685
193	158	4100	4500	-400
194	159	3845	4500	-655
195	160	5649	4500	1149
196	161	6810	4500	2310
197	162	4117	4500	-383
198	163	5385	4500	885
199	164	6035	4500	1535
200	165	6029	4500	1529
201	166	3746	4500	-754
202	167	9700	9000	700
205	170	4000	4000	0
206	171	2075	2000	75
207	172	1009	2000	-991
208	173	891	2000	-1109
209	174	2067	2000	67
Total:		114511	106500	8011

Project Number: GM 89.3.2
Project Title: Gypsy Moth Rearing FY89: Subsidized Research and Otis Projects
Report Period: October 1, 1988 - September 30, 1989
Report Type: Final
Project Leader: G. Bernon

Figure 1 provides a summary of diet expenses for in-house projects in FY89. This excludes labor and some miscellaneous expenses but does provide an indication of resources allocated per project (see Figure 1, percent allocation per project). Twenty-five research groups were also subsidized with gypsy moths reared at Otis (Figure 2). Expenses incurred for providing egg masses to research groups are included in Colony Production. Support for the University of Massachusetts (Dr. Joe Elkinton) is listed separately.

A limited number of pupae was also provided (see Figures 1 and 2). During the first quarter of the fiscal year, egg mass quality was adversely affected by Abnormal Performance Syndrome (APS) as explained in GM 88.3.1. A temporary measure to circumvent this problem was to supply neonate larvae in diet-filled cups to researchers pending improvement of egg mass quality. Therefore, larvae expenses are also listed separately in Figure 1 and total larvae shipped are summarized in Figure 2. While shipping larvae was necessary to continue support to cooperators, it proved to be cumbersome and expensive and thus only egg masses will be supplied in FY90 unless quality is adversely affected by APS.

Figure 1. Cost of diet and rearing containers for in-house projects and subsidized researchers.

		[1]/Colony	[2]/Ins	[3]/SMT	[4]/QC	[5]/F1-10K Eggs	[6]/F1-SMT	[7]/UMass	[8]/Strain Dev't	[9]/Morph.	Misc.	Cooperator Larvae	Cooperator Pupae	Total
Oct - July	# Cups	60954	29560	47034	12967	15750	6600	7040	6258	792	17097	7697	1807	213,555.55
	Cost[a]	$7,070.66	$3,428.96	$5,455.94	$1,504.17	$1,827.00	$765.60	$816.64	$725.93	$91.87	$1,983.25	$892.85	$209.57	$24,772.44
Aug - Sept	# Cups	14400	5520	18360	2760	0	38640	0	5520	0	0	1000	1000	87200
	Cost[b]	$1,684.80	$645.84	$2,148.12	$322.92	$0.00	$4,520.88	$0.00	$645.84	$0.00	$0.00	$117.00	$117.00	$10,202.40
Oct - Sept	Misc. Supplies	$192.06	$89.39	$166.80	$40.08	$40.08	$115.96	$17.91	$30.09	$2.02	$43.50	$22.15	$7.16	$767.21
Overtime per Project							$761.76							$761.76
Total Cost per Project FY1989		$8,947.52	$4,164.19	$7,770.87	$1,867.17	$1,867.08	$6,164.20	$834.55	$1,401.86	$93.89	$2,026.76	$1,032.00	$333.73	$36,503.82
% Total Alloc. of $15,000		24.51%	11.41%	21.29%	5.12%	5.11%	16.89%	2.29%	3.84%	0.26%	5.55%	2.83%	0.91%	100.00%
		$3,676.68	$1,711.13	$3,193.17	$767.25	$767.21	$2,532.97	$342.93	$576.05	$38.58	$832.83	$424.06	$137.13	$15,000.00
Trays		1021.30	475.31	886.99	213.13	213.11	703.60	95.26	160.01	10.72	231.34	117.80	38.09	4166.67

[a] cost per cup = $ .116
[b] cost per cup = $ .117

[1]/	Colony Production (including egg masses for subsidized researchers)	G. Bernon
[2]/	Insecticide Unit	W. McLane
[3]/	Sterile Male Technique	V. Mastro
[4]/	Quality Control	J. A. Tanner
[5]/	F1 - 10K Eggs	V. Mastro
[6]/	F1 - Sterile Male Technique	V. Mastro
[7]/	University of Massachusetts	J. Elkinton
[8]/	Strain Development	R. Hansen
[9]/	Morphometrics	P. Kingsley

Figure 2. Otis Methods Development Center shipping records, fiscal year 1989

Cooperator	Agency	Lifestage	Rate	Last Shipment	Next Shipment	Egg Masses To Date	Larvae To Date	Pupae To Date
1. A. Adamson	ESPRO	eggs & larvae	1000/wk	JD270	JD275	7360	53850	2825
2. P. Barbosa	Univ. MD.	eggs	25/mo	JD268	JD298	853	1500	0
3. J. Brezner	SUNY	eggs	50/mo	JD212	JD242	410	1000	0
4. R. Cardé	Univ. MA	eggs & larvae	10/wk	JD268	JD275	1585	43600	2710
5. R. Charlton	Cornell Univ.	eggs & larvae	40/mo	JD268	JD298	1570	34575	450
6. J. Cunningham	Forest Pest Mgt.	egg masses	irregular	JD226	Pending	1077	0	0
7. B. Dahr	IL. Nat. Hist.	eggs	10/mo	JD268	JD298	713	4750	0
8. L. Dapsis	Ocean Spray	larvae	irregular	JD249	Pending	0	25500	0
9. R. Deans	Cape Cod Research	larvae	irregular	JD227	Pending	0	2500	0
10. J. Elkinton	Univ. MA	eggs & larvae	variable	JD268	Pending	1327	25940	6420
11. A. Hajek	USDA, ARS	eggs & larvae	20/2 wks	JD268	JD282	1203	22500	0
12. J. Harnstad	Univ. WI	eggs	irregular	JD265	Pending	200	0	0
13. A. Iskra	U.S. Forest Svc.	larvae	300/wk	JD268	JD275	457	2000	0
14. D. Leonard	Univ. MA	pupae	irregular	JD188	Pending	0	0	500
15. R. Lindroth	Univ. WI	eggs	irregular	JD01	Pending	117	0	0
16. C. ODell	U.S. Forest Svc.	eggs	irregular	JD269	Pending	176	0	0
17. G. Prestwich	SUNY	larvae	1 tray/2 wk	JD255	JD268	0	4010	900
18. P. Schaefer	USDA, BIRL	pupae	irregular	JD257	Pending	80	0	600
19. J. Schultz	PA State Univ.	eggs & larvae	25/mo	JD268	JD298	1137	31600	0
20. S. Shives	VA Dept. Ag.	eggs & larvae	32-120/mo	JD269	JD299	1034	9500	0
21. D. Smitley	MI State Univ.	eggs	10/mo	JD136	JD167	95	1000	0
22. R. Taylor	OH State Univ.	eggs	8/mo	JD136	JD167	97	1000	0
23. M. Ticehurst	Natl. GM Mgt. Grp.	eggs & larvae	120/mo	JD269	Pending	4811	43600	750
24. R. Vogt	Yale Univ.	larvae	1 tray/mo	JD269	JD275	0	7040	2850
25. W. Yendol	PA State Univ.	eggs	120/mo	JD128	JD159	789	1000	0
			Total for Fiscal Year 1989:			28091	316465	18005

Project Number: GM 89.3.3
Project Title: Evaluation of near-wild gypsy moth strains
Report Period: December 1, 1988 to September 30, 1989
Report Type: Interim
Project Leader: R.W. Hansen, with the assistance of V. Baker

Introduction

Within a laboratory rearing scenario, an insect strain can be defined as an intraspecific group from a distinct geographic region that, presumably, is genetically similar. Strains are presumed to differ genetically; phenotypically, they differ physiologically but usually not morphologically. Many insect rearing operations maintain a primary, production strain for most or all rearing objectives. Additional strains may be maintained to meet different production objectives, to provide a source of genetic diversity through outcrossings with a production strain, and to serve as "backups" or replacements in the event of "failure" of a production strain.

The OMDC utilizes the "New Jersey" strain, currently in its 35th generation, as its production strain. A number of "near-wild" gypsy moth strains have been maintained at the OMDC for various lengths of time and for a number of initial purposes. (A near-wild strain is defined as one maintained for at least one previous generation in culture.) However, little or no quantitative data exists on the "performance" (growth, development, and survival) of these strains under laboratory conditions. Additionally, we wished to reduce the number of strains propagated in order to maintain strains of relatively high "quality" and to minimize costs.

The objective of this study was to evaluate physiological "performance" of near-wild gypsy moth strains currently available at Otis, and compare performance to that of the "New Jersey" strain (control). Using these data, a scheme to objectively rank these strains would be developed, so that the "best" strains could be selected for continued propagation.

Methods

Ten near-wild gypsy moth strains were available for study.

Strain	Code	Collection site	Gen. in culture
Ashumet	AS	MA	5
Canadian-1	C1	Nova Scotia	1
Clay	CL	WV	4
Maine	ME		6
North Mtn.	NM	WV	4
Pine Knoll	PK	NC	3
Rhode Island	RI		2
"Nondiapause"	ND	RI	3
Sleepy Creek	SC	WV	5
Yards Creek	YC	NJ	>6?
New Jersey (control) [Subcolony (PEID) 113]	NJ		33

For each strain, egg masses were removed from cold storage (5-7°C) after ca. 150 days exposure. Six to 12 egg masses were pseudorandomly selected from those available and disinfected in a 0.1% sodium hypochlorite solution (5 min., with a distilled water rinse). Masses were placed in Petri dishes and held at 25°C, >80% RH. Egg masses were held at 27°C, >75% RH until hatch. First instars were used in "experimental" or "production" rearings, as described below.

I. Experimental evaluations

As eggs hatched, first instars were placed individually in 60-ml (2 oz) clear plastic cups with 15 ml of diet, or in groups of 10 in 200-ml (6 oz) translucent plastic cups with ca. 85 ml of diet. One hundred individual rearings and 30 group rearings (n=300 larvae) were established for each strain. First instars were taken in roughly equal numbers from each egg mass and equally represented at least two days of hatch.

Containers were maintained at 24-26°C and 50-60% RH under a 16:8 hr (L:D) photoperiod. Rearing containers were examined at eight and 21 days after introduction, when survival and larval stadium were recorded. Fresh diet cups were provided as necessitated by larval feeding, desiccation, or fungal contamination. As larvae approached pupation, containers were examined daily. Pupae were collected, sexed, and pupation dates recorded. After 24 hours, pupae were weighed (to the nearest 0.1 mg) and pupal deformities, if present, recorded (see Report GM 89.3.5 for description of pupal abnormalities and their occurrence). Pupae were then reared individually until adult eclosion.

Data were analyzed using two- or three-way ANOVA, with strain, rearing container (group or individual), and sex, when appropriate, as the independent variables. The Student-Newman-Keuls (SNK) multiple range test was used to detect significant differences among means. ANOVA procedures were executed with $\alpha=0.05$.

II. Production rearings

For each strain, remaining first instars from "experimental" egg masses, and larvae hatching from additional surface-sterilized masses, were reared in groups of 10 in 200-ml cups. Cups were examined at 35 days, and roughly every other day until 45 days, after introduction. Pupae were collected and groups of 25 females and 30 males (or as many of each sex available) placed in 4.2-l (1 gal) paper containers lined with removable kraft paper. Containers were then held at 27°C and >60% RH for ca. 40 days to permit adult eclosion, mating, oviposition, and egg embryonation. After 40 days, the dead adults were vacuumed off and the kraft paper removed. The egg masses in each container were counted, cut from the paper background, and stored in groups of 30-50 in 530-ml (16 oz) paper cups. Egg mass containers were held for 10 days at 10°C and then placed into cold storage at 5°C. Thus, production rearings provided the next generation's egg masses. However, data from these rearings will not be discussed at this time.

Results - experimental evaluations

Table 1 shows that relatively little mortality occurred by 8 or 21 days into the larval stage. Most mortality occurred later in the larval stage for most strains - i.e. larvae that failed to pupate. Generally, adults eclosed from most (>75%) pupae. NJ (control) insects exhibited the highest generational survival (L_1 to adult).

Mean larval stadium at day 8 (Table 2) varied significantly among strains ($F=195.9$, $P<0.001$) and between rearing containers (group>individual; $F=29.2$, $P<0.001$). The strain/container interaction term was also significant ($F=5.4$, $P<0.001$). RI and ND larvae exhibited the greatest mean stadium, but differences among strains are small and probably not biologically significant. AS, CL, PK, SC, YC, and NJ (control) also exhibited means of 1.5 or greater (group rearings). At 21 days (Table 2), mean larval stadium also differed significantly among strains ($F=208.7$, $P<0.001$) and between rearing types (group> individual; $F=41.7$, $P<0.001$); the interaction between strain and container was also significant ($F=20.2$, $P<0.001$). NJ (control) mean stadium was greatest, though ND, RI, and AS (group) also exhibited mean stadia of 4.0 or greater.

Larval stadium data from day 8 and day 21 can also be expressed as diversity values, i.e. the distribution of observations among categories (here, stadia). Table 3 shows Shannon's diversity index (H) and that diversity expressed as a proportion of maximum possible diversity (J). The smaller the H and J values, the less variable is larval development.

The length of the larval stage (Table 4) varied significantly among strains ($F=193.7$, $P<0.001$) and between sexes (♀>♂; $F=191.3$, $P<0.001$), but not between rearing containers ($F=1.1$, $P>0.10$). Strain/sex and strain/container interaction terms were also significant. NJ (control) insects exhibited the shortest and least variable larval stage and NM the longest. It is interesting to note that, on average, larvae of most near-wild

strains required more than 35 days to complete development. Undoubtedly, prolonged female development reflects the occurrence of a sixth stadium among many near-wild females. This stadium has virtually disappeared among NJ insects.

Pupal weight data are summarized in Table 5. Mean pupal weight varied significantly among strains ($F=38.4$, $P<0.001$), between sexes (♀>♂; $F=5347$, $P<0.001$), and between rearing containers (individual>group; $F=8.0$, $P<0.01$). Interaction terms are also significant. NJ (control) pupal weights were the least variable (as measured by CV). Female pupal weights can describe insect "fitness" because of their relationship with fecundities.

For all strains, the length of the pupal stage averaged about 14 days for males and 12 days for females. Mean pupal stage length varied significantly between sexes ($F=1741$, $P<0.001$) but not between rearing container ($F=2.0$, $P>0.10$). Mean pupal stage length varied significantly among strains ($F=16.0$, $P<0.001$). However, among-strain differences are probably not biologically significant, since the range of means for either sex did not exceed 0.5 days.

Finally, I attempted to objectively rank these strains based on their physiological performance. This was done using the following equation:

$$\text{"SCORE"} = (A\text{-}CV_A) + 0.33(B\text{-}CV_B) + ((100/C)\text{-}2*CV_C) + ((100/D)\text{-}2*CV_D) + (E\text{-}CV_E) + 2*F$$

where A = mean stadium, day 8
B = mean stadium, day 21
C = mean larval stage (days), females
D = mean larval stage (days), males
E = mean female pupal weight
F = generational survival (L_1 to adult)
CV = coefficient of variability (SD/mean) of above

Strains are ranked from lowest to highest score in Table 6. A relative score, based on the highest value (NJ), ranks the strains on a 0.0 - 1.0 basis. NJ (control) insects exhibited the highest ranking, due primarily to their lower mean larval stage duration (both males and females) and higher generational survival. RI, ND, YC, AS, and YC are the highest-ranked of the near-wild strains (scores >0.75). However, there were not enough YC L_1 to initiate production rearings, and this strain has become "extinct". Using a relative score of 0.75 as a selection point, the remaining four strains (AS, ND, RI, and SC) were maintained for further propagation and evaluation.

Table 1. Percent survival of near-wild strains from L_1 establishment to various points in the life cycle. Numbers in parentheses indicate percent survival from preceding stage.

			Survival (%) to:			
			Larvae			
Strain	Type	N (start)	Day-8	Day-21	Pupae	Adults
AS	GR	300	88	87 (98)	67 (77)	65 (97)
	Ind.	100	92	89 (97)	25 (28)	20 (80)
C1	GR	300	92	84 (91)	26 (30)	22 (84)
	Ind	100	94	86 (91)	35 (41)	32 (91)
CL	GR	300	93	86 (92)	45 (53)	38 (84)
	Ind	100	95	90 (95)	59 (65)	57 (97)
ME	GR	299	84	77 (92)	38 (49)	34 (91)
	Ind	100	96	80 (83)	14 (17)	11 (79)
ND	GR	300	99	98 (99)	58 (59)	44 (76)
	Ind	100	100	100 (100)	85 (85)	68 (80)
NM	GR	300	91	87 (96)	36 (41)	28 (79)
	Ind	100	89	88 (99)	48 (54)	35 (73)
PK	GR	300	95	89 (94)	14 (15)	9 (63)
	Ind	100	95	92 (97)	28 (30)	22 (79)
RI	GR	300	97	95 (99)	82 (86)	78 (96)
	Ind	100	95	95 (100)	87 (92)	86 (99)
SC	GR	300	97	95 (98)	58 (61)	54 (92)
	Ind	100	92	88 (96)	59 (67)	42 (71)
YC	GR	300	96	95 (99)	63 (66)	57 (91)
	Ind	100	93	92 (99)	60 (65)	58 (97)
NJ	GR	300	98	96 (98)	90 (93)	82 (92)
	Ind	100	100	98 (98)	97 (99)	93 (96)

Table 2. Mean larval stadium[a] at 8 and 21 days after L_1 introduction.

Strain	Type	Day 8 n	Day 8 Mean stadium: $\bar{X}$	Day 8 2SE	Day 8 CV(%)	Day 21 n	Day 21 Mean stadium: $\bar{X}$	Day 21 2SE	Day 21 CV(%)
AS	GR	265	1.6	0.1	31	260	4.0	0.1	29
	Ind	92	1.2	0.1	34	89	2.3	0.3	63
C1	GR	277	1.1	0.0	32	253	2.7	0.2	47
	Ind	94	1.1	0.1	26	86	2.4	0.3	57
CL	GR	279	1.5	0.1	35	258	3.6	0.2	40
	Ind	95	1.5	0.1	34	90	3.7	0.3	39
ME	GR	251	1.2	0.1	33	230	3.3	0.1	35
	Ind	96	1.1	0.1	27	80	2.1	0.2	54
ND	GR	297	2.0	0.0	7	294	4.6	0.1	12
	Ind	100	2.0	0.0	11	100	4.7	0.1	11
NM	GR	273	1.1	0.0	32	261	2.3	0.2	56
	Ind	89	1.1	0.1	30	88	2.4	0.3	50
PK	GR	284	1.5	0.1	34	266	3.0	0.2	40
	Ind	95	1.4	0.1	35	92	3.0	0.3	43
RI	GR	290	2.0	0.1	27	286	4.5	0.1	19
	Ind	95	2.0	0.1	21	95	4.7	0.1	14
SC	GR	290	1.8	0.1	39	285	4.0	0.1	32
	Ind	92	1.6	0.1	36	88	3.7	0.3	37
YC	GR	288	1.5	0.1	33	285	3.9	0.1	29
	Ind	93	1.3	0.1	35	92	3.7	0.2	31
NJ	GR	294	1.9	0.1	21	289	4.9	0.1	10
	Ind	100	1.9	0.1	17	98	4.9	0.1	9

[a] SNK test - day 8: (RI ND) > NJ > SC > (AS CL YC PK) > (ME NM C1)
day 21: NJ > (ND RI) > (SC YC) > (CL AS) > (ME PK) > C1 > NM

Table 3. Diversity values for day-8 and day-21 larval stadia data. Indices are derived from group rearing data

	Day-8		Day-21	
Strain	H[a]	J	H	J
AS	0.6928	0.6306	1.2950	0.8046
C1	0.4377	0.3985	1.5188	0.9437
CL	0.7449	0.6780	1.4783	0.9185
ME	0.5048	0.4595	1.4680	0.9121
ND	0.0988	0.0900	0.7762	0.4823
NM	0.4204	0.3827	1.4283	0.8875
PK	0.7119	0.6480	1.4741	0.9159
RI	0.8023	0.7303	0.8992	0.5587
SC	1.0424	0.9489	1.2199	0.7579
YC	0.6931	0.6309	1.3124	0.8154
NJ	0.5433	0.4946	0.3498	0.2173

[a] $H = - \Sigma (p_i * \log p_i)$; $J = H/H_{max}$

Table 4. Length of larval stage (L_1 to pupation) for near-wild strains.

Strain	Sex	Type	n	Mean[1](d)	2SE	CV(%)	Min.	Max.
AS	M	GR	113	36.0	0.8	12	30	46
		Ind	14	37.9	4.1	20	31	56
	F	GR	87	38.9	0.8	10	31	55
		Ind	10	45.0	3.9	14	39	58
C1	M	GR	55	40.6	1.6	15	31	58
		Ind	18	45.2	4.7	22	35	66
	F	GR	22	44.8	3.0	16	35	60
		Ind	17	47.9	3.5	15	39	61
CL	M	GR	63	36.2	2.4	26	28	69
		Ind	30	33.9	1.9	15	30	53
	F	GR	73	39.0	1.6	18	31	60
		Ind	29	40.4	2.8	19	33	61
ME	M	GR	64	36.8	1.5	16	29	57
		Ind	5	39.0	5.2	15	33	55
	F	GR	49	41.3	1.5	12	32	59
		Ind	8	44.1	6.5	21	38	59
ND	M	GR	82	33.6	0.9	12	29	46
		Ind	39	31.6	1.2	12	27	43
	F	GR	92	39.1	1.1	13	30	52
		Ind	42	37.9	1.7	15	29	52
NM	M	GR	64	50.2	3.0	24	32	69
		Ind	26	46.9	4.4	24	31	69
	F	GR	44	52.1	3.0	19	35	68
		Ind	22	50.6	3.2	15	39	64
PK	M	GR	28	43.1	3.6	22	34	68
		Ind	17	41.0	3.8	19	31	65
	F	GR	13	48.4	3.1	12	38	57
		Ind	11	40.1	2.4	10	36	51
RI	M	GR	137	33.1	0.7	12	26	46
		Ind	49	32.7	1.3	14	28	52
	F	GR	108	36.4	1.1	16	29	61
		Ind	38	35.2	1.1	9	28	44

Table 4 - cont'd.

Strain	Sex	Type	n	Mean[1](d)	2SE	CV(%)	Min.	Max.
SC	M	GR	72	33.6	0.8	10	29	55
		Ind	23	36.7	2.4	15	31	52
	F	GR	101	38.1	0.8	11	31	49
		Ind	36	41.1	1.4	10	34	53
YC	M	GR	92	34.8	0.8	11	29	46
		Ind	43	38.5	2.5	21	30	63
	F	GR	96	37.1	0.7	10	29	50
		Ind	17	38.5	2.2	12	33	48
NJ	M	GR	135	28.0	0.3	6	23	35
		Ind	48	27.9	0.8	10	24	43
	F	GR	125	29.8	0.4	8	28	43
		Ind	49	30.6	1.0	11	28	48

[1] SNK test: NM>(PK C1)>ME>(CL AS SC YC ND)>RI>NJ

Table 5. Pupal weight summary for near-wild strains.

Strain	Sex	Type	n	Mean[1](g)	2SE	CV(%)	Min.	Max.
AS	M	GR	113	0.5612	0.0201	20	0.2341	0.7067
		Ind	14	0.5163	0.0816	30	0.2305	0.6674
	F	GR	85	2.0721	0.1220	27	0.7088	3.4510
		Ind	10	1.6451	0.4117	39	0.2132	2.2501
C1	M	GR	54	0.5338	0.0501	34	0.1983	0.9853
		Ind	18	0.5658	0.0733	27	0.2298	0.7687
	F	GR	22	2.0502	0.4290	49	0.3230	3.5803
		Ind	17	1.4793	0.2759	38	0.4174	2.2635
CL	M	GR	63	0.6351	0.0494	31	0.1260	0.9229
		Ind	30	0.6680	0.0490	20	0.3679	0.8870
	F	GR	72	2.3242	0.2019	37	0.3944	3.8618
		Ind	29	2.2447	0.2573	31	0.6683	3.2567
ME	M	GR	64	0.6325	0.0459	29	0.1513	1.0168
		Ind	5	0.6009	0.1282	23	0.4190	0.7941
	F	GR	48	2.2238	0.2102	35	0.5814	3.5667
		Ind	8	1.7694	0.5487	44	0.5055	2.3700
ND	M	GR	82	0.6314	0.0417	30	0.2289	0.9787
		Ind	39	0.7144	0.0403	18	0.3443	0.8615
	F	GR	90	1.7761	0.1293	34	0.3836	3.0820
		Ind	42	2.0062	0.1439	23	0.9447	2.9280
NM	M	GR	64	0.4835	0.0558	46	0.1000	0.9532
		Ind	26	0.4746	0.0597	32	0.2457	0.7053
	F	GR	44	1.5090	0.2317	51	0.2458	3.2494
		Ind	22	1.4273	0.2399	39	0.3796	2.5495
PK	M	GR	28	0.4230	0.0640	40	0.0569	0.7338
		Ind	17	0.5211	0.0724	29	0.1959	0.7233
	F	GR	13	1.2816	0.4710	66	0.4660	3.6810
		Ind	11	1.3122	0.2291	29	0.7110	1.8735
RI	M	GR	137	0.6422	0.0245	22	0.2174	1.0051
		Ind	49	0.7040	0.0348	17	0.3743	0.9625
	F	GR	108	2.1100	0.1560	38	0.4072	3.8316
		Ind	38	2.9636	0.1736	18	1.3454	3.7902

Table 5. - cont'd.

Strain	Sex	Type	n	Mean[1](g)	2SE	CV(%)	Min.	Max.
SC	M	GR	73	0.6127	0.0352	25	0.1930	0.8747
		Ind	23	0.6146	0.0550	22	0.3939	0.9443
	F	GR	101	1.7369	0.1396	40	0.4941	3.9089
		Ind	36	1.7550	0.1700	29	0.7996	2.7698
YC	M	GR	91	0.6580	0.0277	20	0.2611	0.9138
		Ind	43	0.6939	0.0419	20	0.3401	1.0645
	F	GR	95	2.2930	0.1208	26	0.7953	3.5150
		Ind	17	2.3057	0.1356	12	1.6766	2.6919
NJ	M	GR	131	0.6273	0.0158	14	0.2530	0.8496
		Ind	48	0.7077	0.0282	14	0.4279	0.8917
	F	GR	121	2.2502	0.0749	18	0.7078	3.1710
		Ind	49	2.3314	0.1269	19	0.6350	3.1903

[1] SNK test: (CL NJ YC RI ME ND SC AS) > (C1 NM) > PK

Table 6. Scores used to rank near-wild strains. Data were derived from group rearings (see text for details).

Strain	Score	Relative score
AS	10.54	0.77
C1	7.64	0.56
CL	9.35	0.68
ME	8.99	0.66
ND	10.76	0.79
NM	6.00	0.44
PK	6.51	0.48
RI	11.85	0.87
SC	10.33	0.76
YC	10.71	0.78
NJ	13.65	1.00

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Introduction

The previous report describes evaluation efforts with near-wild gypsy moth strains maintained in laboratory culture for at least one (parental) generation. This report summarizes efforts to similarly evaluate "wild" strains, initiated with egg masses collected from feral populations. The objective of this experiment was to evaluate survival and physiological performance of feral gypsy moth strains under artificial rearing conditions, and compare their performance to that of the standard lab ("New Jersey") strain. Hopefully, this effort will aid in our understanding of gypsy moth "domestication" (adaptation to artificial rearing environments), and will permit the initiation of "new" near-wild strains for future evaluation and propagation.

Methods

Gypsy moth egg masses (P_1 generation) were collected from various North American localities in late 1988 or early 1989. Collection localities and strain designations are described below:

Strain code	Locality	Collection date
WV	West Virginia (Morgan Co.)	March 1989
VA	Virginia (Shenandoah Co.)	Feb. 1989
MI	Michigan (Isabella Co.)	March 1989
C2	Nova Scotia (New Minas)	Fall 1988
UT	Utah (Salt Lake Co.)	Dec. 1988
PA	Pennsylvania (Elk Co.)	April 1989

Lab-strain ("New Jersey", hereafter referred to as NJ; subcolony (PEID) 248, G_{34}) egg masses provided control insects.

For each strain, ca. 200 ml of loose egg mass fragments were surface-disinfected in a 0.1% sodium hypochlorite solution, rinsed in water, and air-dried. Eggs were held in an incubator at 27°C (ca. 80% RH) until hatch. Ten newly-hatched first instars were placed in 200-ml plastic cups containing 85 ml of standard artificial diet. A total of 60 cups (600 larvae) were established for each strain.

NPV-induced larval mortality was noted among MI, VA, and WV cups. Thus, a second cohort was established for these strains. About 200 ml of egg mass fragments were disinfected as above, air-dried, and dehaired. Dehaired eggs were disinfected a second time. A surfactant, Tween® (1% v/v), was added to the sodium hypochlorite solution used for these eggs. Results for this cohort are indicated by a "B" following the strain code (MI, VA, WV) in the following data summaries.

Cups were maintained at 27° and ca. 60% RH under a 14:10 (hr L:D) photoperiod provided by fluorescent lights. Cups were examined at 8 and 21 days after establishment, and the stadium of each surviving larva recorded. Incidences of viral-induced larval mortality were noted; cups were discarded if several virus-killed cadavers were discovered. Fresh diet cups were provided as necessary. Cups were examined daily with

the onset of pupation. All pupae were collected and sexed and the pupation date recorded. Pupae were weighed (to the nearest 0.1 mg) and examined for pupal deformities (see Report GM 89.3.5 for details) 24-48 hrs after collection. Collected pupae were maintained individually in clear plastic cups until adult eclosion, and eclosion dates were recorded.

Additional larvae were established to provide egg masses for future experimentation. These "production" rearings were examined every other day beginning about 28 days after establishment, and all pupae were collected. Results from these rearings will also not be discussed in this report.

Data analysis procedures employed were the same as those described in the preceding report.

Results

Table 1 summarizes insect survival for wild strains and the NJ control. For most strains, most mortality occurred after day 21; i.e. larvae failed to pupate. Exceptions to this generalization were C2 insects, which suffered fairly high mortality prior to day 8 and exhibited relatively high survival thereafter, and NJ (control) insects, which exhibited high survival values through adult eclosion. In general, the double disinfection procedure ("B") did not greatly reduce NPV-induced losses, as measured by number of cups discarded. Survival to the adult stage was not recorded for MI, WV, or VA because pupae were collected for "production" matings.

Mean stadium at day 8 differed significantly among strains (Table 2; $F = 133.4$, $P < 0.001$). PA and WV values were not different from the control (NJ) mean. Generally, UT and C2 development were similar with relatively low variability (i.e. CV values). Mean stadium also differed among strains at day 21 (Table 2; $F = 106.5$, $P < 0.001$). NJ larvae exhibited the greatest mean stadium, but this value was not significantly different from PA, C2, UT, or WV-"B" means. Table 3 expresses stadium distribution data in terms of Shannon's diversity index. The smaller the H and J values, the less variable is larval development. At day 8, PA exhibited the lowest diversity values, while NJ (control) and PA diversity indices were smallest at day 21.

The length of the larval stage (L_1 introduction to pupation) was significantly longer for females than males (Table 4; $F = 764.1$, $P < 0.001$) and varied significantly among strains ($F = 384.5$, $P < 0.001$). NJ (control) larvae developed fastest and VA insects the slowest, with the remaining strains intermediate. The analysis was repeated with $\log_{10}$-transformed data, and results did not change. Mean larval stage durations for a number of strains were less than 35 days, unlike results reported with "near-wild" strains. Though wild strain larvae commonly exhibited more than five stadia, the higher rearing temperature (27°) undoubtedly accelerated development.

Table 5 summarizes wild strain pupal weight data. Means varied between sexes (♀ > ♂; $F = 4339$, $P < 0.001$) and among strains ($F = 31.1$, $P < 0.001$). Generally, VA insects produced the smallest pupae; no clear differences existed among the remaining wild strains and the NJ controls. The use of transformed data did not affect these conclusions.

The relative "performance" of wild strains was also assessed using the "ranking equation" previously described (survival to the pupal stage was substituted for survival to the adult stage). Scores are summarized in Table 6. NJ (control) insects again achieved the highest ranking, primarily due to shorter mean development time and higher survival. Using a relative score of 0.75 as a "cut-off", C2, PA, UT, and WV were selected for future production and evaluation.

Conclusions

Ten near-wild and six wild gypsy moth strains were evaluated under laboratory rearing conditions. Four of each type were maintained for future propagation and evaluation, based on their growth, development, and survival. These eight strains will be similarly evaluated over a second generation, and four strains will be selected for future work.

Ultimately, I hope this effort will yield two near-wild strains of "known" quality, able to survive and develop under artificial rearing conditions yet differing genotypically and phenotypically from the "New Jersey" strain. Future work will also include yearly evaluation of several "new" feral strains initiated from various North American localities. This continuing effort could "discover" new strains to replace one or both of the near-wild strains as well as providing feral insects for research projects. Additionally, interstrain crosses between the "New Jersey" and various wild and near-wild strains have been initiated.

Near-wild strains will be maintained as alternate strains for different production and research objectives, on a relatively small scale (hundreds of egg masses) and over a limited season (ca. six-month egg mass availability). They will also provide a source of genetic diversity via outcrosses with the New Jersey strain. However, these strains are not presently envisioned as replacements for the New Jersey strain.

Table 1. Percent survival of wild strains from L_1 establishment to various points in the life cycle. N (starting population) = 600 for all strains.

Strain	% survival[a]: Larvae Day 8: Virus[b]	Day 8: Survival	Day 21: Virus	Day 21: Survival	Pupae Virus	Pupae Survival	Adults
C2	0	**68**/-	3	**63**/66 (93)	0	**55**/59 (87)	**44**/47 (80)
MI	21	**57**/87	- - - - - - - - - - - Data not recorded - - - - - - - - - - -				
MI-"B"	0	**91**/-	35	**34**/82 (38)	1	**5**/12 (14)	---
PA	0	**94**/-	1	**92**/94 (98)	1	**70**/73 (76)	**64**/66 (90)
UT	0	**97**/-	0	**95**/- (98)	0	**73**/- (77)	**59**/- (80)
VA	0	**97**/-	20	**60**/90 (62)	2	**30**/48 (50)	---
VA-"B"	0	**96**/-	14	**71**/93 (74)	1	**47**/62 (66)	---
WV	0	**96**/-	5	**87**/95 (91)	0	**69**/76 (80)	**59**/64 (85)
WV-"B"	0	**95**/-	15	**72**/96 (76)	0	**56**/75 (78)	---
NJ (control)	0	**98**/-	0	**93**/- (95)	0	**89**/- (95)	**84**/- (95)

a Survival values reported as: **aa**/bb, where **aa** is survival when all mortality agents are considered, and bb is survival when estimated NPV mortality is removed. Values is parentheses indicate survival from preceding stage.

b Number of cups (10 larvae/cup) discarded due to NPV contamination

Table 2. Mean stadium of wild strain larvae at 8 and 21 days after L_1 introduction. Means followed by the same letter are not significantly different (SNK test; $\alpha = 0.05$).

	Day 8				Day 21			
		Mean stadium:				Mean stadium:		
Strain	n	$\bar{X}$	2SE	CV(%)	n	$\bar{X}$	2SE	CV(%)
C2	406	2.5 b	0.1	23	379	4.7 c	0.1	11
MI	340	1.8 e	0.1	41		----------		
MI-"B"	548	1.9 d	0.1	39	206	4.3 e	0.1	18
PA	566	2.6 a	0.1	20	553	4.8 ab	0.1	9
UT	585	2.4 b	0.1	23	571	4.8 bc	0.0	9
VA	582	1.9 d	0.1	31	362	4.0 f	0.2	20
VA-"B"	576	2.3 c	0.1	26	426	4.5 d	0.1	14
WV	575	2.6 a	0.1	21	521	4.4 e	0.1	15
WV-"B"	569	2.3 c	0.1	25	434	4.7 c	0.1	11
NJ (control)	588	2.6 a	0.1	24	560	4.9 a	0.1	10

Table 3. Diversity values for day-8 and day-21 larval stadia data.

Strain	Day 8		Day 21	
	H[a]	J	H	J
C2	0.8324	0.7577	0.6326	0.3931
MI-"B"	1.0627	0.9673	1.0334	0.6421
PA	0.7245	0.6595	0.4676	0.2906
UT	0.8229	0.7491	0.5607	0.3484
VA	0.9137	0.8317	1.1550	0.7176
VA-"B"	0.8847	0.8053	0.8738	0.5429
WV	0.7650	0.6963	0.9475	0.5887
WV-"B"	0.8660	0.7883	0.6950	0.4319
NJ	0.7879	0.7172	0.2525	0.1569

[a] $H = - \Sigma (p_i * \log p_i)$; $J = H/H_{max}$

Table 4. Length of larval stage (L_1 to pupation) for wild strains.

Strain	Sex	n	Mean (d)	2SE	CV(%)	Min.	Max.	Sig.[a]
C2	M	138	31.4	0.7	15	25	51	f
	F	163	36.4	0.8	14	27	51	
MI	M	31	32.1	0.9	7	29	37	g
	F	14	34.3	1.0	5	29	37	
MI-"B"	M	6	46.7	5.9	15	41	61	b
	F	23	42.6	1.7	10	38	50	
PA	M	156	31.4	0.8	16	25	54	ef
	F	267	36.6	0.6	13	27	54	
UT	M	239	34.6	0.6	13	27	53	c
	F	200	40.8	0.6	10	31	51	
VA	M	97	45.4	1.9	21	27	70	a
	F	85	49.2	2.0	19	31	67	
VA-"B"	M	171	34.5	0.9	17	27	57	cd
	F	109	39.8	1.2	15	28	59	
WV	M	229	32.8	0.7	17	25	68	def
	F	187	38.4	0.7	13	28	57	
WV-"B"	M	205	33.4	0.7	15	27	55	cde
	F	134	40.3	1.0	14	29	57	
NJ (control)	M	262	24.9	0.4	12	21	44	h
	F	272	27.1	0.4	12	24	47	

a Means for strains followed by same letter not significantly different (SNK test: $\alpha = 0.05$); mean values for females significantly greater than for males, regardless of strain.

Table 5. Pupal weight data summary for wild strains.

Strain	Sex	n	Mean (g)	2SE	CV(%)	Min.	Max.	Sig.[a]
C2	M	138	0.6694	0.04	35	0.1101	1.2687	d
	F	160	2.3406	0.14	38	0.3202	4.2183	
MI	M	29	0.7433	0.04	15	0.4441	0.8934	cd
	F	13	2.8503	0.38	32	1.0572	4.0735	
MI-"B"	M	6	0.3299	0.14	53	0.1280	0.6373	cd
	F	23	1.5984	0.31	46	0.4013	3.0769	
PA	M	155	0.6495	0.03	33	0.1539	1.0180	d
	F	263	1.9295	0.10	44	0.3793	4.2761	
UT	M	239	0.6233	0.02	26	0.0970	1.0442	bc
	F	200	1.9444	0.13	46	0.4497	4.5296	
VA	M	97	0.3950	0.04	47	0.1252	0.9249	a
	F	85	1.4217	0.14	47	0.2748	3.4702	
VA-"B"	M	170	0.5924	0.04	40	0.1079	1.0718	b
	F	109	1.9854	0.18	48	0.3898	4.1603	
WV	M	229	0.6448	0.03	31	0.1151	1.1910	c
	F	185	2.1241	0.13	43	0.2667	3.7884	
WV-"B"	M	203	0.6596	0.03	36	0.1216	1.2051	bc
	F	133	2.0635	0.15	43	0.2951	3.6117	
NJ (control)	M	258	0.6994	0.01	16	0.2174	1.0020	d
	F	271	2.2397	0.05	18	0.6170	3.2290	

a Means for strains followed by same letter not significantly different (SNK test: $\alpha = 0.05$); mean values for females significantly greater than for males, regardless of strain.

Table 6. Ranking of wild strains based on survival and physiological performance.

Strain	Score[a]	Relative score[b]
C2	12.27	0.82
MI	9.86	0.66
MI-"B"	8.24	0.55
PA	12.23	0.81
UT	11.54	0.77
VA	8.18	0.54
VA-"B"	11.00	0.73
WV	12.05	0.80
WV-"B"	11.58	0.77
NJ	15.01	1.00

a See preceding report for details.
b Score/highest score (NJ = 15.01)

Project Number: GM 89.3.5
Project Title: Pupal deformities among laboratory-reared gypsy moths
Report Period: December 1, 1988 to September 30, 1989
Report Type: Interim
Project Leader: R.W. Hansen

Introduction

Morphological "abnormalities" have long been noted among diet-reared gypsy moth pupae, but little quantitative information on their occurrence is available. This report includes data from several experiments that document the incidence of pupal deformities, and relate their frequency to other physiological factors and rearing conditions.

I. Incidence of pupal deformities and their relationship to development time and pupal weight.

Methods

Gypsy moths from ten "near-wild" strains (at least one previous generation in culture) and the standard "New Jersey" lab strain (G_{33}) were reared in two types of containers. Groups of 10 newly-hatched first instars were reared to pupation in 200-ml translucent plastic cups (n=300 L_1/strain); cups contained ca. 85 ml of artificial diet. Other larvae were reared individually in 65-ml clear plastic cups with ca. 15 ml diet (n=100 L_1/strain). Larvae were maintained at 25°C and 40-60% RH under a 16:8 hr (L:D) photoperiod.

Pupae were collected daily and weighed within 24-48 hrs. Pupae were examined and the occurrence of one or more of the following "pupal deformities" noted for each.

Code	Description
BA	"Banding" - light-colored cuticular band on ventral surface of first abdominal segment; apparently nonsclerotized
BL	"Light" banding - as above, but an arbitrary designation as "less severe" banding (narrower width); included with above in data analysis
SV	"sunken face" - a sunken area or "pit" between distal portions of forewings and mouthparts; often underlain by nonsclerotized cuticle
MW	"misshapen wings" - malformed or unequal-sized pupal forewings
DA	"detached antennae" - most of the antennal sheaths detached from pupal thorax
HB	"humpbacked" - distinct humpbacked appearance of dorsal thorax; pupae usually appear curved when viewed laterally
GA	"gross abnormalities" - generally, large areas of nonsclerotized cuticle and missing or deformed appendages on thorax, presence of larval structures (legs, head capsule), or an incomplete larval/pupal ecdysis; may include one or more of the above categories

Collected pupae were reared individually until adult eclosion.

I reared a second cohort of gypsy moths to pupation, including six feral strains (field-collected egg masses) and the "New Jersey" (control) strain (G_{33}). Groups of ten first instars were reared in 200-ml cups as previously described (n=600 L_1/strain); no larvae were reared individually. Cups were maintained at 27°C

and 50-60% RH under a 14:10 hr photoperiod. Pupae were collected daily, and deformities recorded, as described above.

Additional details regarding rearing and data collection procedures may be found in the preceding reports.

Results

Pupal deformities were common among "near-wild" and lab-strain females but relatively infrequent among males (Table 1). "Banding" (BA and BL) was the most common abnormality among female pupae, an observation somewhat obscured by the small numbers of individually-reared pupae surviving for some near-wild strains (Table 1). Similar pupal deformity patterns were observed among wild strains and their "New Jersey" counterparts (Table 2). The occurrence of banding among near-wild, wild, and lab (NJ) strains is further illustrated in Figs. 1 and 2.

Relationships between banding frequency and female developmental time or pupal weight were derived from a near-wild (ND) and wild (UT) strain and the two NJ cohorts. Banding generally increased in frequency with increased pupal weight (Fig. 3), but became less frequent with longer development time (Fig. 4). However, these associations are themselves obscured by significant negative linear relationships between pupal weight and development time (pooled data for near-wild strains: $Y = 3.941 - 0.050X$, $r = 0.50$; for wild strains: $Y = 4.099 - 0.055X$, $r = 0.50$).

The presence of pupal deformities had little direct effect on adult eclosion. When data for near-wild and NJ insects were pooled, banded male and female pupae yielded adults as frequently as "normal" pupae (Table 3). However, adults were unlikely to eclose from pupae with "gross abnormalities". Adults were not examined, so potential effects of pupal deformities on adult morphology cannot be estimated.

II. Effects of diet and rearing environment on the incidence of pupal deformities

Methods

Beginning in May 1989, "New Jersey" larvae (G_{34}) were reared from the first stadium to pupation on black or red oak foliage, in an outdoor insectary or in free-standing screen cages. Groups of 100-200 larvae were provided with ca. 0.5-m foliated oak branches kept in water-filled flasks. Fresh branches were provided every 5-7 days.

Other "New Jersey" larvae were reared, in groups of ten, in 200-ml cups containing ca. 85 ml artificial diet. Cups were maintained in an outdoor insectary under ambient conditions. Many of these larvae were later transferred to oak foliage for various experiments, but some were reared to pupation on diet.

On June 28, 1989, several hundred "feral" fifth instars were collected from a mixed oak stand near Freetown, MA. Larvae were reared to pupation on cut white and black oak branches in large outdoor cages.

Female pupae from these three rearing treatments were collected every other day and examined for deformities, as previously described. Freetown (feral) pupae were also weighed.

Results

Deformities among foliage-fed "New Jersey and Freetown (FT) and insectary-reared, diet-fed NJ pupae are summarized in Table 4. Figure 5 illustrates banding patterns among female pupae; data for lab-reared NJ pupae (25° and 27°C) are included for comparison. Deformities generally, and banding specifically, were virtually absent among foliage-reared pupae. Diet-fed female pupae reared outdoors exhibited deformities much less frequently than their lab-reared counterparts.

The only other physiological data recorded from these insects were weights for Freetown (FT) pupae; mean values for males (0.4951 g, $s = 0.1002$) and females (1.2194 g, $s = 0.2858$) compare to means of 0.6273 g ($s = 0.0902$) and 2.2502 g ($s = 0.4120$) for NJ males and females, respectively (group-reared, 25°C).

Discussion

Pupal deformities were common among lab-reared female gypsy moths but rare among males. The occurrence of pupal abnormalities has no apparent genetic basis, since similar patterns were exhibited among feral, near-wild, and lab strains (all presumably genotypically "distinct"). It is not clear how the incidence of "banding" and other deformities relates to larval development time or pupal weight.

Nutritional, habitat, and environmental factors may be responsible for pupal deformities among lab-reared gypsy moths. The scarcity of deformities among foliage-reared pupae suggests that artificial diet lacks certain "essential" nutrients or possesses deleterious compounds. Dahlman and Rosenthal (1975) described sublethal effects of the arginine homolog, L-canavanine (a nitrogen storage molecule in many legumes) on *Manduca sexta*. These include malformed pupae and poor sclerotization, perhaps akin to "banding". A proline homolog, L-azetidine-2-carboxylic acid (found in several plant families and in a marine alga) induced deformities and lack of sclerotization among *Heliothis zea* pupae (Adeyeye and Blum 1989). Perhaps gypsy moth diet ingredients contain low levels of these, or similar, compounds.

Alternatively, oak branches or rearing cages may provide a more favorable habitat for pupation than plastic cups. Perhaps larvae and prepupae require favorable "substrates" for a successful larval/pupal ecdysis. Density effects ("crowding" in the small cups?) may also be important.

The reduced frequency of deformities among diet-fed pupae reared outdoors suggests that the laboratory rearing environment (thermoperiod? photoperiod? humidity?) increases the incidence of banding and other abnormalities.

Most pupal abnormalities have no direct impact on adult eclosion. However, insects in these experiments were handled very carefully. Malformed (particularly "banded") pupae are more fragile and may be damaged (e.g. rupture of nonsclerotized cuticle) during the handling and mechanical transport procedures of pupal harvest. "Wounded" pupae may produce fewer adults. In any event, only about 80% of collected female pupae produce an egg mass in typical production mating containers. Pupal deformities should be of concern even if they have no demonstrable impact on rearing "productivity". Such abnormalities occur very rarely among feral, foliage-fed gypsy moths. Pupal deformities thus represent a comparative reduction in the quality of mass-reared insects, either directly or as "indicators" of other, less-visible physiological changes.

Future research efforts will attempt to isolate and clarify mechanism(s) contributing to pupal abnormalities among mass-reared gypsy moths. These will also quantify the impact of deformities on egg mass yield in the mass-rearing environment.

LITERATURE CITED

Adeyeye, O.A. and M.S. Blum. 1989. Inhibition of growth and development of *Heliothis zea* (Lepidoptera: Noctuidae) by a nonprotein imino acid, L-azetidine-2-carboxylic acid. Environ. Entomol. 18: 608-611.

Dahlman, D.L. and. G.A. Rosenthal. 1975. Non-protein amino acid - insect interactions. I. Growth effects and symptomatology of L-canavanine consumption by tobacco hornworm, *Manduca sexta* (L.). Comp. Biochem. Physiol. 51(A): 33-36.

Table 1. Proportionate occurrence of malformed pupae and those pupae with no morphological deformities among "near-wild" and NJ strains (group and individually reared). See text for explanation of deformity categories.

				Proportion of pupae[a]: Deformity category:							
Strain	Type	Sex	n	BA	BL	SV	MW	DA	HB	GA	None
AS	Gr	M	113	0.02	0.05	0.0	0.0	0.01	0.0	0.02	0.90
		F	85	0.59	0.20	0.12	0.13	0.0	0.0	0.0	0.20
	Ind	M	14	0.07	0.07	0.0	0.0	0.0	0.0	0.0	0.86
		F	11	0.18	0.18	0.27	0.0	0.09	0.0	0.09	0.27
C1	Gr	M	54	0.0	0.02	0.0	0.0	0.02	0.0	0.0	0.98
		F	22	0.45	0.27	0.0	0.04	0.0	0.0	0.0	0.27
	Ind	M	18	0.0	0.06	0.0	0.0	0.0	0.0	0.0	0.89
		F	17	0.0	0.12	0.06	0.06	0.0	0.12	0.06	0.65
CL	Gr	M	63	0.02	0.05	0.02	0.0	0.02	0.0	0.02	0.92
		F	74	0.61	0.18	0.11	0.13	0.01	0.0	0.05	0.15
	Ind	M	30	0.0	0.03	0.0	0.0	0.0	0.0	0.0	0.97
		F	29	0.17	0.45	0.17	0.0	0.0	0.03	0.0	0.38
ME	Gr	M	64	0.19	0.14	0.0	0.09	0.0	0.0	0.04	0.61
		F	48	0.79	0.06	0.19	0.10	0.06	0.0	0.06	0.08
	Ind	M	6	0.17	0.17	0.0	0.0	0.0	0.0	0.0	0.50
		F	8	0.50	0.37	0.12	0.37	0.12	0.0	0.0	0.0
ND	Gr	M	82	0.07	0.05	0.01	0.01	0.05	0.0	0.0	0.85
		F	90	0.61	0.23	0.17	0.20	0.02	0.0	0.06	0.06
	Ind	M	41	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.95
		F	44	0.23	0.41	0.43	0.09	0.20	0.20	0.0	0.09
NM	Gr	M	67	0.0	0.0	0.0	0.03	0.03	0.01	0.0	0.92
		F	49	0.20	0.10	0.02	0.06	0.04	0.04	0.02	0.61
	Ind	M	25	0.0	0.0	0.04	0.04	0.04	0.0	0.0	0.88
		F	22	0.04	0.04	0.23	0.04	0.18	0.04	0.0	0.50
PK	Gr	M	29	0.0	0.03	0.0	0.10	0.14	0.0	0.03	0.72
		F	13	0.23	0.08	0.23	0.0	0.08	0.23	0.08	0.31
	Ind	M	17	0.06	0.0	0.0	0.0	0.06	0.0	0.0	0.94
		F	12	0.08	0.08	0.25	0.08	0.0	0.0	0.0	0.42

Table 1. - cont'd.

Strain	Type	Sex	n	BA	BL	SV	MW	DA	HB	GA	None
RI	Gr	M	137	0.0	0.01	0.01	0.01	0.01	0.01	0.0	0.96
		F	108	0.25	0.27	0.11	0.09	0.05	0.0	0.02	0.40
	Ind	M	50	0.02	0.0	0.02	0.0	0.0	0.0	0.0	0.98
		F	37	0.27	0.46	0.19	0.11	0.05	0.03	0.0	0.16
SC	Gr	M	72	0.01	0.04	0.01	0.0	0.3	0.0	0.03	0.87
		F	101	0.58	0.21	0.04	0.09	0.08	0.0	0.06	0.12
	Ind	M	23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
		F	36	0.25	0.44	0.36	0.11	0.05	0.03	0.03	0.17
YC	Gr	M	91	0.01	0.05	0.01	0.0	0.03	0.0	0.0	0.92
		F	95	0.29	0.31	0.06	0.07	0.07	0.0	0.03	0.32
	Ind	M	43	0.0	0.07	0.0	0.0	0.0	0.0	0.0	0.93
		F	17	0.23	0.23	0.23	0.18	0.23	0.29	0.0	0.23
NJ	Gr	M	130	0.01	0.11	0.01	0.0	0.0	0.0	0.0	0.88
		F	122	0.62	0.28	0.06	0.06	0.02	0.01	0.02	0.08
	Ind	M	48	0.0	0.10	0.0	0.0	0.0	0.0	0.0	0.90
		F	49	0.24	0.31	0.26	0.04	0.06	0.0	0.04	0.22

[a] Sum of proportions may be greater than 1.0 since some pupae had more than one deformity

Table 2. Proportionate occurrence of malformed pupae and those pupae with no morphological deformities among "wild" and NJ strains (group reared). See text for explanation of deformity categories.

			Proportion of pupae[a]: Type of deformity:							
Strain	Sex	N	BA	BL	SV	MW	DA	HB	GA	Normal
C2	M	138	0.04	0.03	0.0	0.04	0.04	0.0	0.0	0.88
	F	160	0.52	0.09	0.19	0.09	0.01	0.0	0.04	0.22
MI	M	29	0.03	0.03	0.0	0.0	0.07	0.0	0.0	0.90
	F	13	0.92	0.0	0.0	0.0	0.08	0.0	0.0	0.08
PA	M	155	0.02	0.01	0.0	0.0	0.02	0.0	0.01	0.93
	F	263	0.42	0.23	0.03	0.11	0.05	0.0	0.02	0.28
UT	M	239	0.01	0.02	0.0	0.03	0.02	0.0	0.01	0.92
	F	200	0.46	0.20	0.08	0.21	0.05	0.01	0.06	0.19
VA	M	170	0.06	0.08	0.01	0.02	0.06	0.0	0.03	0.76
	F	109	0.49	0.27	0.06	0.11	0.05	0.0	0.08	0.15
WV	M	229	0.03	0.03	0.03	0.06	0.04	0.0	0.05	0.81
	F	185	0.68	0.11	0.19	0.08	0.10	0.01	0.03	0.11
NJ	M	258	0.02	0.01	0.0	0.01	0.01	0.0	0.02	0.94
	F	271	0.55	0.17	0.01	0.08	0.04	0.0	0.0	0.22

[a] Sum of proportions may be greater than 1.0 since some pupae had more than one deformity

Table 3. Effects of pupal "banding" and gross abnormalities on subsequent adult eclosion. Data are pooled from all near-wild strains and associated lab-strain controls, reared in the laboratory.

Sex	Deformity	N	N eclosing	% eclosing
Females	Banding (BA and BL)	524	468	89
	"Gross" abnormalities	27	6	22
	"Normal"	139	121	87
Males	Banding (BA and BL)	64	53	83
	"Gross" abnormalities	10	0	0
	"Normal"	673	609	90

Table 4. Relative occurrence of various deformities among foliage-reared and diet-fed "New Jersey" and feral (FT) pupae, reared in outdoor cages.

Strain	Sex	N	Proportion of pupae[a]: Type of deformity: BA	BL	SV	MW	DA	HB	GA	Normal
NJ (Foliage)	F	154	0.01	0.01	0.0	0.02	0.02	0.02	0.0	0.93
FT (Foliage)	M	143	0.01	0.01	0.01	0.0	0.01	0.0	0.01	0.96
	F	385	0.0	0.02	0.02	0.01	0.01	0.01	0.0	0.94
NJ (diet)	M	119	0.0	0.0	0.0	0.0	0.01	0.0	0.0	0.99
	F	140	0.06	0.20	0.02	0.03	0.01	0.01	0.02	0.67

[a] Sum of proportions may be greater than 1.0 since some pupae had more than one deformity

Figure 1. Frequency of banding among male and female pupae of "near-wild" strains.

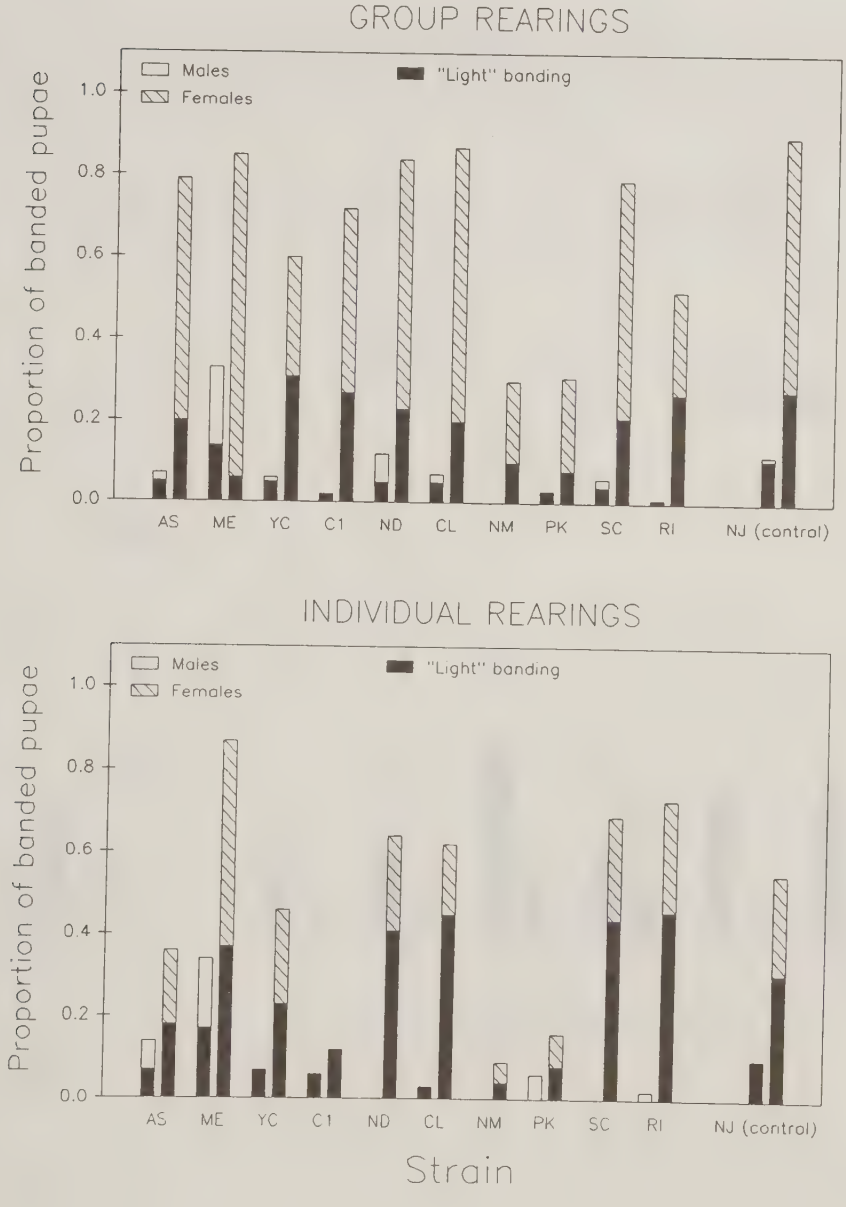


Figure 2. Frequency of banding among male and female pupae of "wild" strains.

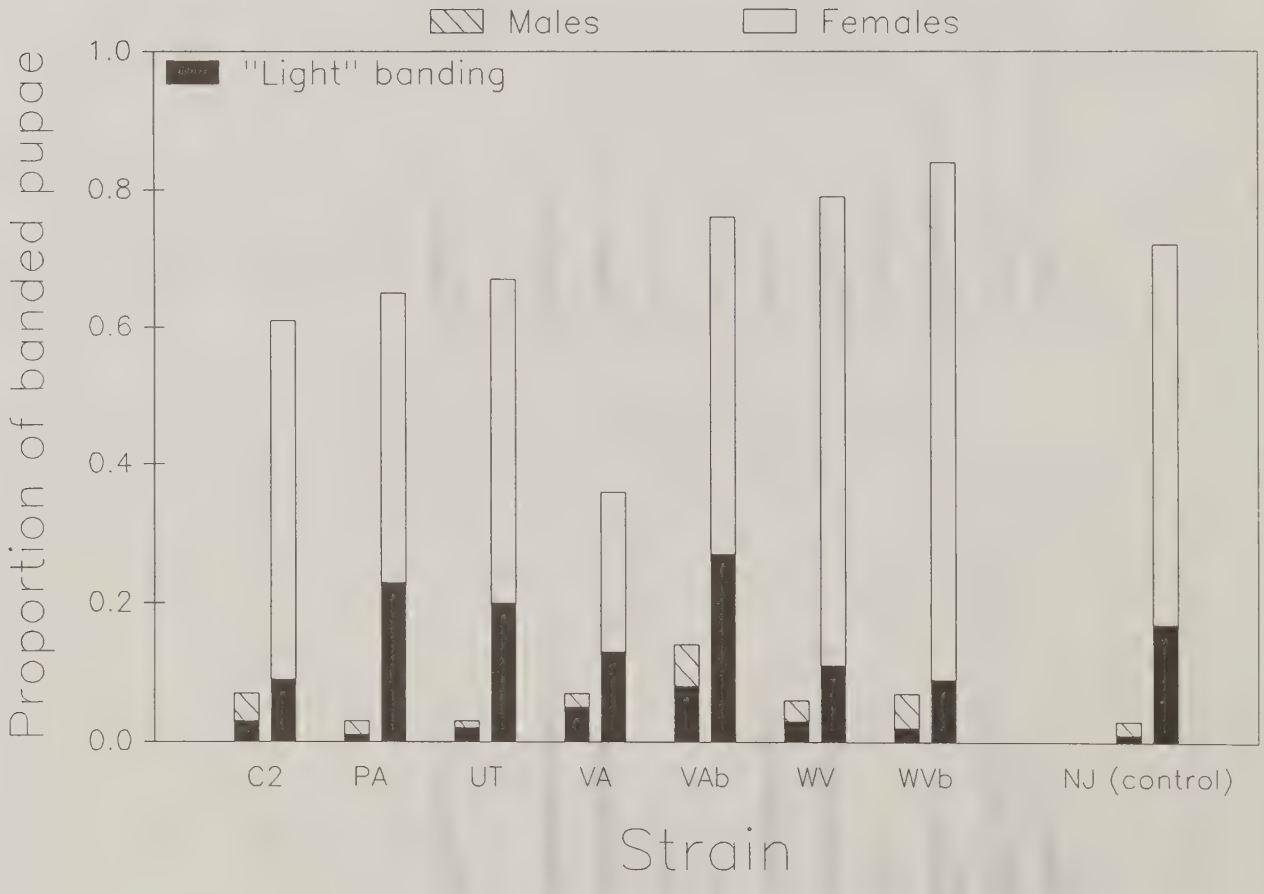


Figure 3. Frequency of banding among female pupae as a function of pupal weight (group-reared larvae).

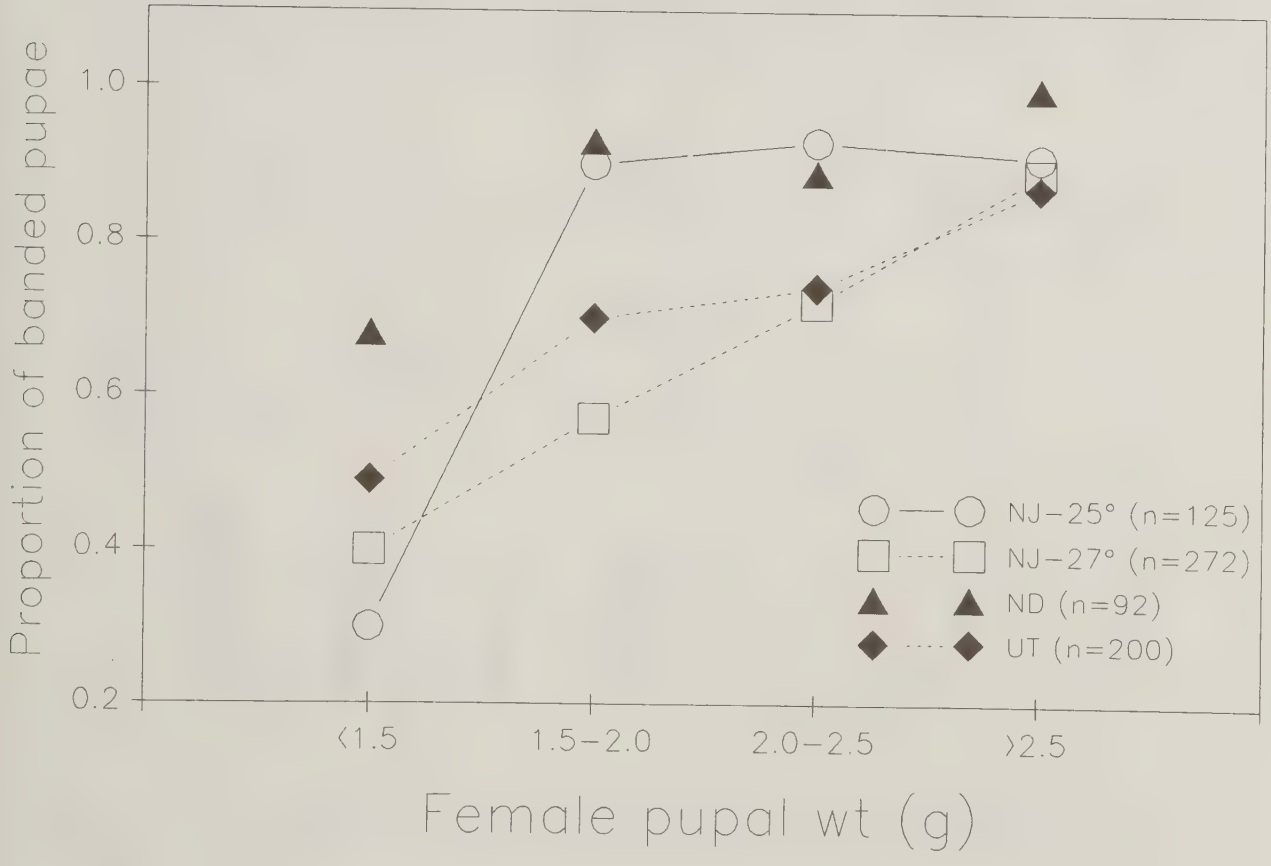
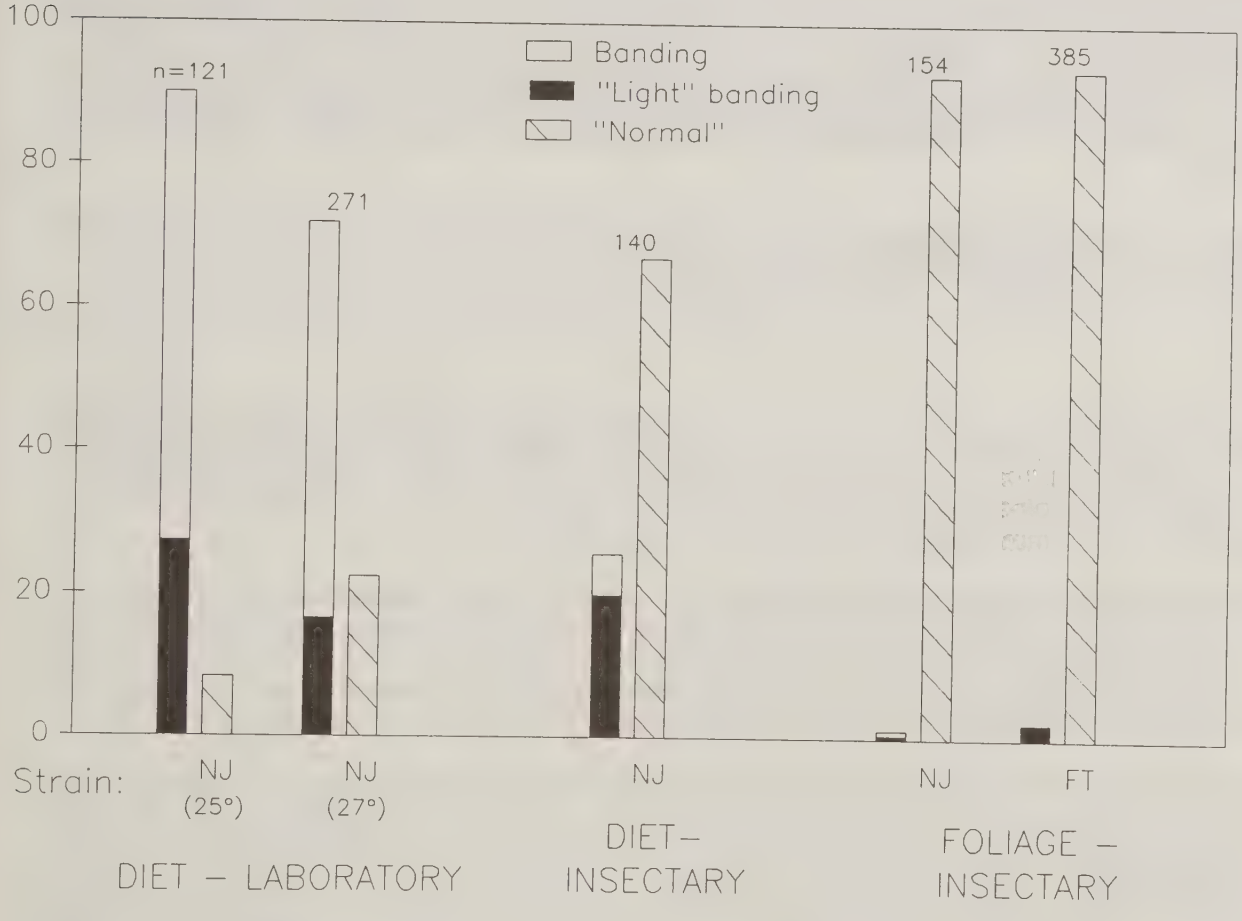


Figure 4. Frequency of banding among female pupae as a function of larval development time (group-reared larvae).

Figure 5. Frequency of banding among female pupae reared on diet or oak foliage, in the laboratory or an insectary.



Project Number: HWA 89.1.1
Project Title: Cold-hardiness of the hemlock woolly adelgid
Report Period: January 1, 1989 to May 31, 1989
Report Type: Interim
Project Leader: R.W. Hansen

Introduction

The hemlock woolly adelgid (*Adelges tsugae* Annand) (Homoptera: Adelgidae), or HWA, is an introduced insect that has become a pest in the eastern United States on eastern hemlock, *Tsuga canadensis.* The insect feeds primarily on young branches and twigs, removing sap and perhaps injecting a toxin. This feeding damage results in disruption of spring bud flush, loss of needles, and death of twigs and branches. Heavy infestations may kill trees (McClure 1987). Presently, the adelgid occurs from Virginia north into Connecticut.

The HWA has a complex life cycle that includes wingless and winged stages (McClure 1989). Winter is spent as immature female nymphs, or sistens, that remain immobile under a waxy secretion. This insect does not undergo a true winter hibernation or diapause, and continues feeding and developing from October to April. The females mature in early spring, and lay about 20 eggs in a woolly ovisac. Based on the absence of winter diapause, and experiences with the closely-related balsam woolly adelgid (*A. piceae*), some have suggested that northward spread of the HWA may be limited by low winter temperatures. Northward spread is a concern because of the potential threat the HWA presents to large, valuable hemlock stands in northern New England.

The objective of this preliminary experiment was to determine the survival of overwintering hemlock woolly adelgids exposed to low temperatures for various lengths of time. This information will be used to determine if a "low lethal" temperature exists, and whether northward spread of the insect will likely continue.

Methods

HWA-infested hemlock branches were collected on March 9, 1989 in Gales Ferry, New London Co., CT. Branches were removed from heavily-infested understory trees 3 m or less in height; overstory species included hemlock, white pine, and oaks. Infested branches were transported to the laboratory, where 0.5-m apical portions of the most heavily-infested were prepared for study.

Fifteen 0.5-m branches were placed in clear plastic bags and stored in chambers maintained at constant 0°C (32°F), -18°C (0°F), -21°C (-6°F) and -23°C (-10°F). The 0°C chamber had fluorescent lighting maintained in a 12:12 hr photoperiod, while the other chambers were not illuminated. Fifteen additional branches were placed in kraft paper bags and kept in any outdoor insectary under ambient environmental conditions. Ambient temperatures averaged 5-10°C daily maxima and 0-5°C daily minima during the study.

Five bagged branches were randomly-selected and removed after three and seven days exposure. Branches were removed from bags, placed in water-filled Aqua-piks®, and held at room temperature for two days prior to examination. For the 3-day observations, branches were inspected from the apex basally until 50 adult females were examined. For 7- and 10-day observations, 10 small twigs were selected and five females examined on each. The waxy covering was removed from each examined female and her vitality assessed by positive hemolymph pressure and movement in response to the touch of a dissecting probe. The presence or absence of eggs was noted for each living female, and the presence or absence of any newly-hatched nymphs ("crawlers") was generally noted for each branch.

Results

No living females were found after three or seven days at -18°C (0°F), -21°C (-6°F), and -23°C (-10°F). However, survival averaged 91.2% and 93.6% after three days at 0°C (32°F) and in the insectary, respectively. After seven days, 94.8% of examined females survived outdoor exposure, while 92.0% of those

maintained at 0°C remained alive. Generally, 27-38% of living females had deposited eggs; crawlers were noted only after 10 days at 0°C (one branch).

Conclusions

Temperatures of -18°C (0°F) and colder apparently killed all overwintering females after at least three days exposure. However, these samples were collected in early March, and there is no evidence that insects collected from November to February will exhibit a similar cold response. One might expect that physiological adaptations to low temperatures would be maximized when such conditions are "typical" (i.e. mid-winter). Additionally, this study provides no evidence for mortality at shorter exposure intervals (i.e several hours). A tree-inhabiting insects would likely experience temperatures of -18°C and colder for relatively short periods (several hours in a given day) during winters in much of New England.

Thus, the results of this preliminary study should be interpreted with caution. Future experiments (1989-1990) should clarify the aforementioned questions.

Acknowledgements

The USDA-FS, FPM office in Durham, NH provided partial funding for this study. I thank Mr. Pete Merrill, Connecticut Dept. of Forestry, for assistance in locating the collection site.

LITERATURE CITED

McClure, M.S. 1987. Biology and control of the hemlock woolly adelgid. Conn. Agric. Expt. Sta. Bull. 851. 9 p.

McClure, M.S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 82: 50-54.

Project Number:	JB 84.1.1
Project Title:	Evaluation of Traps and Other Techniques for Controlling Japanese Beetles In and Around Airports and Nurseries
Report Period:	October 1, 1988 - September 30, 1989
Report Type:	Interim
Project Leaders:	W. H. McLane, T. Ladd and J. Finney

This project continues to be a cooperative effort with Dr. T. Ladd of the Agricultural Research Service, Japanese Beetle Laboratory, Wooster, Ohio. Dr. Ladd prepared the work plan and final report for this work. He and his crew, with the help of personnel from the Otis Methods Development Center, conducted all tree marking, treatments and evaluations.

During 1989, field tests were conducted at Princeton Nurseries in Allentown, New Jersey, to achieve the following objectives:

1. To evaluate bendiocarb in eliminating Japanese beetle larvae from field-grown nursery stock. Tests during 1988 had demonstrated that two or three applications of bendiocarb (mid-July and early August) at 8 lbs AI/acre or three applications (late June, mid-July and early August) at 4 lbs AI/acre could eliminate larvae.

2. To determine if isofenphos (Triumph), diazinon or RH5849 could eliminate larvae from field-grown stock.

In the first test, insecticides, applied uniformly as sprays to the soil in plots surrounding two trees (maple, ash, or locust) six feet apart, were lightly cultivated and watered-in using 1/4 - 1/2 inches of water. There were four replications of each treatment. Insecticides were applied once, twice or three times during the season with three-week intervals between applications. Plots were examined on September 12-13 and data collected by individually digging trees and carefully examining soil surrounding the roots. Overall test procedures were similar to those used in 1988. Insecticides used, dates applied, and rates of application are indicated in Table 1. The data in the table indicate that bendiocarb (three applications at either 4 or 8 lbs AI/acre) and isazophos (2 or 3 applications at 4 lbs AI/acre) were successful in eliminating larvae from trees in the treated plots. No other materials showed such promise.

In a second test, the effects of timing and numbers of applications of bendiocarb (4 lbs AI/acre) on Japanese beetle larvae were determined. Test procedures were identical to those used in Test 1. Data obtained (Table 2) showed that bendiocarb applied following several schedules was able to eliminate larvae from the field-grown stock.

The data from the two tests corroborate those obtained from the tests carried out during 1988, giving further evidence for the usefulness of bendiocarb, applied three times at 3-week intervals during the growing season at either 4 or 8 lbs AI/acre, in eliminating Japanese beetle larvae from field-grown nursery stock.

During September, at grub digging time, soil samples were taken at the 0-3 and 3-6 inch depth for chemical analysis. Samples (42) were sent to the APHIS, National Monitoring and Residue Analysis Laboratory at Gulfport, Mississippi, for analysis. Those results are attached.

Of interest during the 1989 tests were the meteorological conditions that must have influenced the results obtained. In the period between the first and second applications of insecticides, extremely heavy rainfall occurred in the Allentown area. Eleven inches of rain fell during that period with one storm depositing more than seven inches only a few days after the first application was made. During the end of the test period (August-September); however, little rainfall occurred in the test area. When data were collected by digging soil surrounding the roots of the test trees, the soil was generally powder-dry. The effects of this late-summer drought may well be expressed in the low numbers of larvae found in roots of the control plants. Only about 2 larvae per plot (two trees/plot) were recovered from controls during 1989. This compares to 6.8 larvae per plot recovered during 1988 from plots that were closely located to those used during 1989.

The data obtained from the 1989 tests are encouraging, since they not only show that bendiocarb has the potential to eliminate larvae from field-grown nursery stock, but that isazophos also has potential for similar use. We plan to continue work with these two materials during the coming year.

Table 1. Mean numbers of Japanese beetle larvae found in plots (2 trees per plot; 4 replicates) at the Princeton Nurseries, Allentown, N.J., treated with successive applications of insecticides during the summer of 1989[1/].

Insecticide	Lbs AI/Acre	No. of applications		
		1	2	3
RH 5849 2FL	4	0.0	0.3	0.3
Diazinon 4E	10	0.0	0.8	0.5
Bendiocarb 76%WP	4	0.0	0.0	0.0
Bendiocarb 76%WP	8	0.0	0.3	0.0
Isazophos 4E	2	1.8	0.0	1.0
Isazophos 4E	4	2.0	0.0	0.0
Control		2.3	1.0	0.8

1/ 1st application, June 27; 2nd application, July 18; 3rd application, August 8; plots examined September 12-13.

Table 2. Japanese beetle larvae found in soil in plots (2 trees per plot; 4 replicates) at the Princeton Nurseries, Allentown, N.J. Plots treated with bendiocarb 4E, 4 lbs AI/acre during the summer of 1989

Dates applied	Avg. no. grubs/plot
6/27 & 7/18	1.3
6/27, 7/18 & 8/8	0.0
7/18	0.0
7/18 & 8/8	0.0
8/8	0.8
None	1.3

Title: Determination of Organophosphorous Residues in Soil Samples
Analyst: J. Allen
Contributors: D. Lander and T. Malone

Introduction

Twenty-four (24) soil samples (controls and treatments) for the Japanese Beetle Program were received from the Otis Methods Development Center by the National Monitoring and Residue Analysis Laboratory in Gulfport, Mississippi for analysis. These samples were analyzed for Triumph® (18) and Diazinon® (12) content.

Objectives

1. To analyze soil samples for Triumph® and Diazinon® residues. These analyses were accomplished by gas-liquid chromatography (GLC).
2. To confirm positive residues identified through use of dissimilar GLC columns and/or detectors.
3. To further confirm selected positive residues through the use of gas chromatography/mass spectrometry (GC/MS) when necessary.

Methodology

NMRAL analyzed the samples in accordance with the following procedures:

1. Processing Procedure PR0016, "Extraction of Malathion, Guthion, Diazinon®, and/or Isofenphos from Soil or Sediment", June 2, 1987.
2. Processing Procedure PR0073, "Analysis of Triumph® in Soil", February 29, 1988.

Analyses for Malathion were accomplished on a Hewlett-Packard (HP) 5890 Gas Chromatograph equipped with dual nitrogen-phosphorus (NP) detectors using the following instrument operating parameters:

HP5890 - (NMRAL #078)

a. Chromatographic columns:
   30 meter DB-17 megabore column
   30 meter DB-1301 megabore column

b. Carrier gas (both columns) - Helium at 17.0 mL/min

c. Temperatures:
   NP Detectors - 240°C
   Injection Port - 220°C
   Oven - 200°C

d. Detector gass flows - Hydrogen - 3mL/min
   Air - 100 mL/min

Results (see enclosed tables)

Summary

Of the eighteen Triumph® samples analyzed, eleven (11) had residues with a high of 1.06 parts per million (ppm). Of the twelve Diazinon® samples analyzed, six (6) had residues with a high of 1.44 ppm.

Table 1. Residue Results of Diazinon in Soil Samples Received from Princeton Nurseries, Allentown, New Jersey.

Lab Number	Sample Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Diazinon
AA19873	010236	Monmouth, NJ	Soil	1	6/27	09/12/89	0.07
AA19874	010203	"	"	1	6/27	09/12/89	0.11
AA19869	020203	"	"	2	6/27,7/18	09/12/89	0.11
AA19872	020236	"	"	2	6/27,7/18	09/12/89	0.14
AA19870	030203	"	"	3	6/27,7/18,8/8	09/12/89	1.44
AA19871	030236	"	"	3	6/27,7/18,8/8	09/12/89	0.94
AA19880	020703	"	Soil (Control)	0	NA	09/12/89	<0.01
AA19981	020736	"	"	0	NA	09/12/89	<0.01
AA19882	010703	"	"	0	NA	09/12/89	<0.01
AA19883	010736	"	"	0	NA	09/12/89	<0.01
AA19984	030736	"	"	0	NA	09/12/89	<0.01
AA19985	030703	"	"	0	NA	09/12/89	<0.01

Lower limit of detection = 0.01 ppm

Table 2. Residue Results of Triumph in Soil Samples Received From Princeton Nurseries, Allentown, New Jersey

Lab Number	Sample Number	County, State	Type of Sample	No. of Treatments Applied	Treatment Dates	Date Sample Collected	Residues (ppm) Triumph
AA19865	010536	Monmouth, NJ	Soil	1	6/27	09/12/89	<0.01
AA19866	010603	"	"	1	6/27	09/12/89	0.19
AA19867	010636	"	"	1	6/27	09/12/89	0.08
AA19868	010503	"	"	1	6/27	09/12/89	0.01
AA19861	020503	"	"	2	6/27,7/18	09/12/89	0.14
AA19862	020536	"	"	2	6/27,7/18	09/12/89	0.02
AA19863	020603	"	"	2	6/27,7/18	09/12/89	0.24
AA19864	020636	"	"	3	6/27,7/18,8/8	09/12/89	0.58
AA19856	030503	"	"	3	6/27,7/18,8/8	09/12/89	0.33
AA19857	030536	"	"	3	6/27,7/18,8/8	09/12/89	0.18
AA19858	030603	"	"	3	6/27,7/18,8/8	09/12/89	
AA19880	020703	"	Soil (Control)	0	NA	09/12/89	<0.01
AA19881	020736	"	"	0	NA	09/12/89	<0.01
AA19882	010703	"	"	0	NA	09/12/89	<0.01
AA19883	010736	"	"	0	NA	09/12/89	<0.01
AA19984	030736	"	"	0	NA	09/12/89	<0.01
AA19985	030703	"	"	0	NA	09/12/89	<0.01

Lower limit of detection = 0.01 ppm

* U. S. GOVERNMENT PRINTING OFFICE: 1990--757-377/20035